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VOL. 55 WASHINGTON, D. C. JULY 1–DECEMBER 15, 1937

CONTENTS

	Page
Effects of the Alkaloid Nicotine on the Rhythmicity of Isolated Heart Preparations From *Periplaneta americana* and *Prodenia eridania*. Illus. J. FRANKLIN YEAGER and J. B. GAHAN	1
The Beet Leafhopper in the Central Columbia River Breeding Area. Illus. ORIN A. HILLS	21
A Transmissible Mosaic Disease of Cauliflower. Illus. C. M. TOMPKINS	33
Relation of Certain Dietary Essentials to Fertility in Sheep. GEORGE H. HART and ROBERT F. MILLER	47
Selenium in Plants in Relation to Its Occurrence in Soils. Illus. JOHN T. MILLER and HORACE G. BYERS	59
The Susceptibility of Potatoes to the Vein-Banding Virus. Illus. LEON K. JONES and C. L. VINCENT	69
Observations on the Effect of Environmental Conditions on the Structure of the Lateral Roots in Sugar Beet. Illus. ERNEST ARTSCHWAGER	81
Effect of the Dwarf Disease on the Alfalfa Plant. Illus. J. L. WEIMER	87
Inheritance of Resistance to Pythium Root Rot in Sorghum. Illus. D. H. BOWMAN, J. H. MARTIN, L. E. MELCHERS, and JOHN H. PARKER	105
Observations of Pathogenic and Antigenic Effects of *Brucella abortus*, United States Bureau of Animal Industry Strain 19. Illus. C. M. HARING and J. TRAUM	117
Further Studies on the Interrelationship of Insects and Fungi in the Deterioration of Felled Norway Pine Logs. Illus. J. G. LEACH, L. W. ORR, and CLYDE CHRISTENSEN	129
Studies on the Inheritance and Development of Fruit Size and Shape in the Tomato. Illus. A. F. YEAGER	141
An Undescribed Potato Disease in West Virginia. Illus. C. R. ORTON and L. M. HILL	153
A Simplified Method of Constructing Merchantable Board-Foot Volume Tables. Illus. R. H. BLYTHE, Jr	159
Rhynchosporium Scald of Barley, Rye, and Other Grasses. Illus. RALPH M. CALDWELL	175
The Biology of *Platygaster herrickii*, a Parasite of the Hessian Fly. Illus. C. C. HILL and W. T. EMERY	199
Observations on the Biology of the Wheat-Stem Maggot in Kansas. Illus. MERLE W. ALLEN and REGINALD H. PAINTER	215
Comparative Conformation, Anatomy, and Udder Characteristics of Cows of Certain Beef and Dairy Breeds. Illus. W. W. SWETT, FRED W. MILLER, R. R. GRAVES, W. H. BLACK, and G. T. CREECH	239
Growth and Development of *Dictyostelium discoideum* With Different Bacterial Associates. Illus. KENNETH B. RAPER	289
Susceptibility to *Cronartium ribicola* of the Four Principal *Ribes* Species Found Within the Commercial Range of *Pinus monticola*. Illus. J. L. MIELKE, T. W. CHILDS, and H. G. LACHMUND	317
A Microchemical Colorimetric pH Procedure for Differentiating the Telia of *Cronartium ribicola* and *C. occidentale*. RUBY J. ACREE and WARREN H. GOSS	347
Mermis subnigrescens, a Nematode Parasite of Grasshoppers. Illus. J. R. CHRISTIE	353
The Production of Citrus Mottle-Leaf in Controlled Nutrient Cultures. Illus. H. D. CHAPMAN, A. P. VANSELOW, and GEORGE F. LIEBIG, Jr	365
Nutritive Value for Dairy Cows of Alfalfa Hay Injured by Sulphur Dioxide. Illus. O. C. CUNNINGHAM, L. H. ADDINGTON, and LEWIS T. ELLIOT	381
Certain Relationships Between the Calcium and Oxalate Content of Foliage of Certain Forest Trees. ROBERT F. CHANDLER, Jr	393

Page

Potentialities of Eradicant Fungicides for Combating Apple Scab and Some Other Plant Diseases. Illus. G. W. KEITT and D. H. PALMITER 397

The Toxicity of Copper-Lime-Arsenic Mixtures to Certain Phytopathogenic Fungi Grown on Malt Agar Plates. Illus. D. H. PALMITER and G. W. KEITT 439

Restoration of Virulence of Attenuated Curly Top Virus by Passage Through Susceptible Plants. Illus. C. F. LACKEY 453

Biological Value of Casein as a Supplement to the Proteins of Barley in Rations for Pigs. E. H. HUGHES 461

Effect of Protein Deficiency in the Ration on the Amount of Feed Consumed by Lambs. Illus. JOHN I. MILLER 467

Cross Transfer of Mineral Nutrients in the Tobacco Plant. Illus. J. E. MCMURTREY, JR 475

Physiologic Races of *Tilletia tritici* and *T. levis*. H. A. RODENHISER and C. S. HOLTON 483

Fusarium Resistance in Wisconsin All Seasons Cabbage. Illus. L. M. BLANK 497

Factors Affecting Chlorosis in Irrigated Wheat. Illus. D. W. ROBERTSON and ROBERT GARDNER 511

Factors Influencing the Ripening Season of Sour Cherries. Illus. V. R. GARDNER 521

Observations on the Variations in Cyanogenetic Power of White Clover Plants. CHARLES F. ROGERS and O. C. FRYKOLM 533

Macrosporogenesis and Embryo-Sac Development in *Euchlaena mexicana* and *Zea mays*. Illus. D. C. COOPER 539

Distribution and Variation With Maturity of Dissolved Solids, Sucrose, and Titratable Acidity in the Sorgo Stalk. EMIL K. VENTRÉ and S. BYALL 553

Foot Rot of China-Aster, Annual Stock, and Transvaal Daisy Caused by *Phytophthora cryptogea*. Illus. C. M. TOMPKINS and C. M. TUCKER 563

A Formula for Reducing the Computations Necessary to Find the Variance of a Set of Averages. WILLIAM DOWELL BATEN 575

The Uronic Acid Content of the Nitrogen-Free Extract of Feeding Stuffs. G. A. GUANZON and W. M. SANDSTROM 581

The Effect of Mechanical Processing of Feeds on the Mastication and Rumination of Steers. Illus. C. H. KICK, PAUL GERLAUGH, A. F. SCHALK, and E. A. SILVER 587

Relation of Water-Soaked Tissues to Infection by *Bacterium angulatum* and *Bact. tabacum*. Illus. JAMES JOHNSON 599

Effect of Species of Host Plant on Nitrogen Fixation in *Melilotus*. P. W. WILSON, J. C. BURTON, and V. S. BOND 619

The Nature and Interaction of Genes Conditioning Reaction to Rust in Flax. Illus. W. M. MYERS 631

Twig Lesions as a Source of Early Spring Infection by the Pear Scab Organism. Illus. J. R. KIENHOLZ and LEROY CHILDS 667

Physiologic Races of *Ustilago hordei*. V. F. TAPKE 683

Improvements in Determination of Oil Deposit on Sprayed Foliage. L. H. DAWSEY and J. HILEY 693

Interrelationships of Egg Production Factors as Determined for White Leghorn Pullets. I. MICHAEL LERNER and LEWIS W. TAYLOR 703

Comparative Reaction of Oat Varieties in the Seedling and Maturing Stages to Physiologic Races of *Puccinia graminis avenae*, and the Distribution of These Races in the United States. Illus. M. N. LEVINE and D. C. SMITH 713

Quantitative Determination of Copper and Estimation of Other Trace Elements by Spectrographic Methods in Wire Grasses From "Salt Sick" and Healthy Areas. L. L. RUSOFF, L. H. ROGERS, and L. W. GADDUM 731

Factors Influencing the Development of Soggy Break-Down in Apples. Illus. H. H. PLAGGE and T. J. MANEY 739

Some Properties of Potato Rugose Mosaic and Its Components. Illus. DONALD FOLSOM and REINER BONDE 765

Response of Oat Varieties to Different Fertility Levels. Illus. C. A. LAMB and ROBERT M. SALTER 785

Some Effects of Storage Conditions on Certain Diseases of Lemons. Illus. CHARLES BROOKS and LACY P. MCCOLLOCH 795

Page

Production of Synthetic Mycorhiza in the Cultivated Cranberry. Illus. HENRY F. BAIN 811
The Loss of Carotene in Hays and Alfalfa Meal During Storage. EDWARD A. KANE, HERBERT G. WISEMAN, and C. A. CARY 837
A Statistical Study of Sampling in Field Surveys of the Fall Population of the European Corn Borer. Illus. M. T. MEYERS and L. H. PATCH 849
An Example of the Ability of *Ribes lacustre* to Intensify *Cronartium ribicola* on *Pinus monticola*. Illus. J. L. MIELKE 873
Water Soaking of Leaves in Relation to Development of the Blackfire Disease of Tobacco. Illus. E. E. CLAYTON 883
Role of Molybdenum in the Utilization of Ammonium and Nitrate Nitrogen by *Aspergillus niger*. ROBERT A. STEINBERG 891
The Translocation of Derris Constituents in Bean Plants. Illus. ROBERT A. FULTON and HORATIO C. MASON 903
Quantitative Injection and Effects of Nicotine in Insects. Illus. N. E. MCINDOO 909
Resistance of Sheep of Different Breeds to Infestation by *Ostertagia circumcincta*. Illus. M. A. STEWART, R. F. MILLER, and J. R. DOUGLAS 923
Growth Substances in Relation to the Mechanism of the Action of Radiation on Plants. H. W. POPP and H. R. C. MCILVAINE 931
The Carbon Metabolism of *Fusarium lycopersici* on Glucose. Illus. GORDON H. PRITHAM and ARTHUR K. ANDERSON 937

ERRATA AND AUTHORS' EMENDATIONS

Page 12, fourth line of text from bottom, reference (*55*) should be (*33*).
Page 14, line 7, reference (*44*) should be (*41*).
Page 50, line 22, "oat to hay" should be "oat hay to."
Page 85, table 1, under the heading "Frequency of endodermis pattern" the order of Greek letters should be alpha, beta, gamma. Under the heading "Remarks," the Greek letters should be deleted.
Page 358, twenty-fourth line from bottom, delete the word "less" between "has" and "access."
Page 405, line 16, "¾-inch areas" should be "three quarter-inch areas."
Page 407, table 2, items 18 and 19, "L 3/18" should be "L 3/10."
Page 436, reference 21, "1921" should be "1821."
Page 447, legends for figures 2 and 3, "copper, lime" should be "copper-lime."
Page 448, line 3, "copper, lime" should be "copper-lime."
Page 455, figure 2, "1.91" should be "2.17."
Page 455, fourth line from bottom, "13.4" should be "13.4 percent."
Page 513, equation at bottom should read $\frac{0.2.5.55}{1}=\frac{60-X}{X-40}$
$X=49.470$ F.
Page 621, footnote 3, delete "Underlined values-mean of duplicates."
Page 622, line 17, "table 11" should be "table 1."
Page 624, delete footnote 2.
Page 628, line 8, reference "(*12*)" should be "(*11*)."
Page 687, table 1, column 5, the figure "7" under the variety Hannchen, race 2, should be ".7."
Page 700, line 6, insert footnote reference 3 after weight. The footnote reads as follows: "Later work has demonstrated that the method of drying the residues to constant weight is not applicable to all petroleum oils. It has been found that certain ordinarily used petroleum oils are partially volatile when heated at 110° C. for 2 hours in an electric oven, and this loss may in extreme cases amount to as much as 33 percent of oil recovered from extracted foliage. By substituting the method of evaporating at room temperature under 2 to 3 mm vacuum, as described on page 694 (sentence immediately above table 1), no losses occur. However, the methods as described and without any changes, apply in the analysis of fatty oils like peanut, corn, and cottonseed oil, for it has been demonstrated that no losses occur when these oils are heated at 110° for 2 hours in an electric oven."
Page 724, line 10, "III" should be "3."
Page 727, line 2, "rust" should be "oat."
Page 741, legend for figure 1, "See fig. 8" should be "See fig. 7."
Page 750, table 6, Lot No. 8, under 1929-30, 30° F. column, the "0" should be "0.9."
Page 795, footnote 1, "1937" should be "1938."
Page 811, footnote 1, "1937" should be "1938."
Page 818, plate 1, magnification for D, "× 240" should be "× 540."
Page 820, last paragraph, line 4, "enveloping" should be "developing."
Page 823, table 1, Lot No. 2, column 8, under "Soluble starch" the leaders should be "2.0."
Page 917, table 5, mean revival time, "39.5" should be "38.5."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 55 WASHINGTON, D. C., JULY 1, 1937 No. 1

EFFECTS OF THE ALKALOID NICOTINE ON THE RHYTHMICITY OF ISOLATED HEART PREPARATIONS FROM PERIPLANETA AMERICANA AND PRODENIA ERIDANIA[1]

By J. FRANKLIN YEAGER, *senior entomologist*, and J. B. GAHAN, *junior entomologist*, *Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture* [2]

INTRODUCTION

This is a report of experiments performed to detect the effects of different concentrations of nicotine, an alkaloid much used in pharmacological studies, upon the contraction rates of isolated insect heart preparations immersed in a perfusion fluid of known composition and temperature, when the pH values of the fluid, with and without dissolved nicotine, fall within the approximate range of 6.8 to 7.8. The isolated heart preparations are so called to distinguish them from hearts truly isolated from their dorsal body-wall connections. They were obtained from large nymphal and imaginal cockroaches (*Periplaneta americana* L.) and from large larval (fifth and sixth instars) southern armyworms (*Prodenia eridania* Cram.).

METHOD

The method used in these experiments is that already described (*40*)[3] and employed (*41*) to detect the cardiac effects of several thiocyanates. It consists in immersing the dissected cardiac preparation, pinned to a wax strip, in air-stirred perfusion fluid in a glass tube held in a water bath having glass sides, through which the heart is illuminated by a strong beam of light. Fresh perfusion fluid from an aspirator bottle is added by drops to that in contact with the heart preparation. Contraction rate is measured with a stop watch or stop clock by determining the time required for 10 complete contractions to occur and converting to beats per minute. Most of the observed points in the illustrative curves represent the average of five such determinations. When the effect of nicotine upon cardiac rate is to be observed, the wax strip is quickly transferred to a second glass tube holding similar air-stirred perfusion fluid in which is dissolved a known concentration of nicotine, and measurement of contraction rate is continued. This process of applying nicotine to the heart will be referred to in this paper as the transfer or strip-transfer procedure. In many experiments a second transfer was made from the saline-

[1] Received for publication Mar. 1, 1937; issued August, 1937.

[2] The authors are indebted to W. M. Mann, director of the National Zoological Park, Washington, D. C., for supplying cockroaches; to L. D. Goodhue, of the Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, for purifying the nicotine used in these experiments; and to C. E. Schoenhals, of the Bureau of Plant Industry, and Saburo Katsura for the translation of Yokoyama's article.

[3] Reference is made by number (italic) to Literature Cited, p. 16.

nicotine solution back to the original saline perfusion fluid, after the tissues had been in the nicotine solution from 30 to 60 minutes.

Another method of applying nicotine, to be referred to as the nicotine-addition procedure, has also been used. It consists merely in pipettng a known quantity of strong nicotine solution into a known quantity of perfusion fluid about the heart preparation, thus applying a known final concentration of nicotine to the tissues. In this case no addition of fresh perfusion fluid from the aspirator bottle is made. This procedure eliminates the mechanical agitation of the heart preparations that results from the transfer procedure, but it involves the danger of temporarily applying to the tissues a stronger concentration of nicotine than that desired. To eliminate, or at least minimize, this danger, the nicotine was added slowly by drops during a period of several minutes.

The manner of dissecting out the roach heart preparation has already been described (*40*). In these experiments the head of the roach was removed from the preparation by section through the neck. To dissect out the larval heart preparation, the larva is submerged in a dissecting tray of perfusion fluid and fixed, dorsal surface down, to the wax bottom of the tray by a pin thrust through the head. The posterior end of the body is held in position by grasping one of the anal legs with forceps. A longitudinal incision is made with scissors through the body wall along the dorsopleural line from the posterior end to the anterior end of the body just back of the head. The lateral margin of the dorsal lip of the incision is spread laterally and pinned to the wax. A similar incision is made postero-anteriorly along the dorsopleural line of the opposite side, and, after removal of the entire ventral body wall (sterna) and the attached ventral nerve chain, the posterior dorsal lip is spread laterally and pinned. The dorsal body wall (terga) at the anterior end is spread laterally and pinned and, when necessary to increase the visibility of the heart wall, the middle region is also pinned laterally. The gut is sectioned at its pharyngeal and rectal terminations and is carefully removed as its tracheal connections are cut. The anterior end of the preparation is sectioned back of the head and the head removed. Care is taken to disturb the cardiac mechanism as little as possible during the dissection. The cardiac mechanism is thus exposed to view on the inside of the dorsal body wall, forming the isolated heart preparation. As a rule, in a given experiment a single wax strip held one roach heart preparation and one larval heart preparation; the latter was usually prepared first, and the regurgitated and excreted materials were removed by washing with perfusion fluid before the roach dissection was made.

The perfusion fluid was composed of 9.82 g of sodium chloride, 0.77 g of potassium chloride, and 0.5 g of calcium chloride per liter of solution in distilled water. This is approximately one-eleventh Lévy's (*26*) stock solution minus bicarbonate and phosphate. The buffers were eliminated in order that the nicotine salts formed should be mainly the hydrochlorides. The various concentrations of nicotine were prepared by dissolving the purified alkaloid in the saline perfusion fluid and then titrating with 1 N hydrochloric acid or, in the case of the higher nicotine concentrations, with more concentrated acid, until the pH value of the nicotine solution was the same as that

of the saline solution used for perfusion. The pH values of the saline and nicotine-saline solutions were considered equalized when they yielded the same color with universal indicator (British Drug Houses) upon being compared in the depressions of a white porcelain spot plate.

The concentrations of nicotine used in this investigation ranged from 0.0005 to 10.0 percent by volume. Each experiment was carried out at constant temperature, usually at 29.0° C., and at a perfusion fluid pH value within the approximate range of 6.8 to 7.8, as measured colorimetrically with universal indicator.

In many of these experiments the contraction rates observed before application of nicotine and after transfer of the preparations from the saline-nicotine solution to the original saline perfusion fluid can be considered to serve as controls. A number of control experiments, however, were performed in which no nicotine was used, and the strip transfers were made from saline to saline and back to saline again. Control experiments were also performed in which saline containing no nicotine was applied by the addition procedure.

In most of the experiments contraction rates were measured at shorter intervals than the 15-minute periods used heretofore (*40*, *41*), in order to allow detection of initial rate changes of brief duration. From 15 to 30 minutes usually elapsed between the beginning of dissection and the beginning of perfusion.

Approximately 200 cardiac preparations have been utilized in these experiments.

RESULTS

The results described here were obtained by the use of zero (control), 0.0005-, 0.005-, 0.0125-, 0.022-, 0.025-, 0.043-, 0.044-, 0.050-, 0.086-, 0.088-, 0.10-, 0.17-, 0.177-, 0.20-, 0.34-, 0.355-, 0.39-, 0.40-, 0.69-, 0.70-, 0.71-, 0.77-, 1.37-, 1.42-, 1.47-, 2.69-, 2.75-, 2.85-, 5.5-, 5.67-, 5.70-, and 10.0-percent solutions of nicotine. Unless otherwise specified, the following statements refer to these concentrations.

STIMULATORY EFFECTS

The stimulatory effects of nicotine upon the contraction rates of the cockroach and the larval heart preparations have been found to differ. In the case of the larval southern armyworm preparation the stimulatory effect was relatively small whether the nicotine was applied by the addition (figs. 1 and 2) or the strip-transfer procedure (figs. 3, 4, and 5).

Nicotine stimulation of the roach heart preparation, exhibited as an initial sudden but temporary increase in contraction rate, was observed in most of the experiments involving nicotine application by addition (fig. 1). When the strip-transfer procedure was used, however, these initial changes in contraction rate showed more variation. In general it was found that with the highest concentrations no appreciable stimulation occurred (fig. 5, *C*), that with lower concentrations an initial transient stimulation appeared (fig. 4, B_1, *C*) which might be partially obscured by the occurrence of a transient depression (figs. 4, *A*, and 5, *A* and *B*), and that with still lower and the lowest concentrations used an initial depression was usually obtained (fig. 3, *D* and

F). An initial depression also occurred with zero concentration (control experiments) when the transfer procedure was employed (fig. 3, *A*, *B*, and *C*).

Not only was the cockroach heart stimulated initially by nicotine application, but upon the second transfer back to the original saline solution, even after the heart preparation had remained 30 to 60

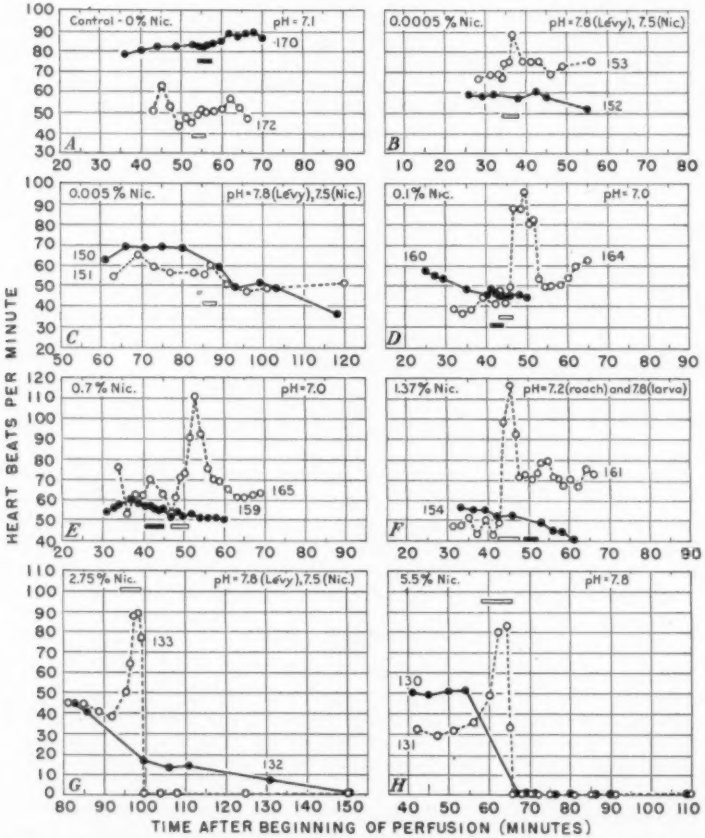

FIGURE 1.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the addition procedure to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Each lettered graph represents an experiment with a different concentration of nicotine or a control. Circles and broken lines represent the roach heart beat and solid dots and lines the larval heart beat. Numbers refer to laboratory number assigned to insect. Small rectangles show the period during which nicotine was being added to the perfusion saline. The pH values indicated are approximate.

minutes in one of the higher nicotine concentrations, it was often left capable of attaining a rate of contraction higher than that exhibited prior to the application of nicotine (figs. 3, *F*, 4, and 5). In many experiments (figs. 3, *E* and *F*, and 4, *A* and *B*), the second transfer was followed by an immediate reversible increase in rate, after which the higher rate level was maintained. In practice, after the second

transfer from nicotine to saline solution, the heart preparation was subject to the action of a very dilute nicotine solution because of the small quantity of the stronger nicotine solution adhering to the strip and attached heart preparation during the transfer.

Initial stimulation of the larval cardiac rate was less marked and of less frequent occurrence than that of the roach heart rate. When the strip-transfer procedure was used, the initial stimulation was usually questionable, but when the addition procedure was used and deter-

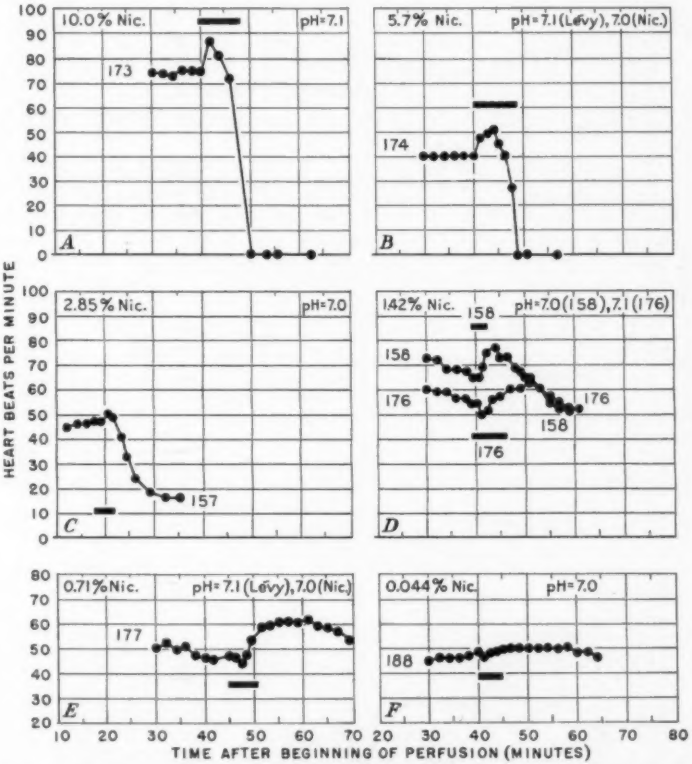

FIGURE 2.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the addition procedure to the perfused isolated heart preparation of *Prodenia eridania*. Each lettered graph represents an experiment with a different concentration of nicotine. Small rectangles show the period during which nicotine was being added to the perfusion saline. Numbers refer to laboratory number assigned to insect. The pH values indicated are approximate.

minations of larval heart rate were made in rapid succession, a small initial increase of rate by the higher nicotine concentrations could be detected (fig. 2). In most of these experiments initial stimulation of larval heart contraction rate has not been readily detected.

When roach or larval heart preparation was perfused with saline containing a low concentration of nicotine, even when initial stimulation was not detectable, often the contraction rate trended upward or at-

tained a higher level and the vigor of contraction appeared somewhat increased, giving the impression that dilute nicotine solutions have

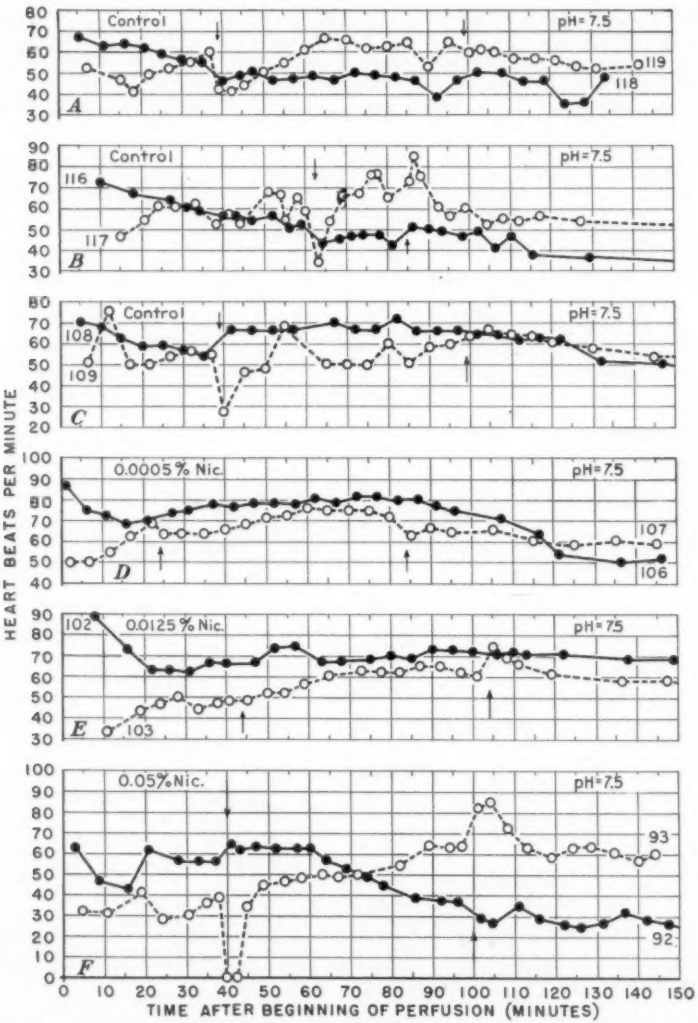

FIGURE 3.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the strip-transfer method to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Circles represent the roach heart beat and solid dots the larval heart beat. Each lettered graph represents an experiment with a different concentration of nicotine or a control. Arrows indicate when the first transfer was made from saline to nicotine-saline solutions and when the second (reverse) transfer was made. Numbers refer to laboratory number assigned to insect. The pH values indicated are approximate.

a beneficial rather than a detrimental effect upon the cardiac mechanism, at least during the period of the experiment.

INHIBITORY EFFECTS

Marked inhibitory effects of nicotine upon the contraction rate of the larval heart were detected in the higher concentrations (figs. 2, *A*, *B*, and *C*, and 5, *B* and *C*). In general this inhibition occurred as a simple decrease in contraction rate to or toward zero, being more rapid and to a lower level the greater the concentration of nicotine (figs. 4 and 5), and it appeared subsequent to initial stimulation (figs. 1, *G* and *H*, 2, *A*, *B*, and *C*, and 4, *C*) when such occurred. Lower nicotine concentrations either had no obvious depressant effect (figs.

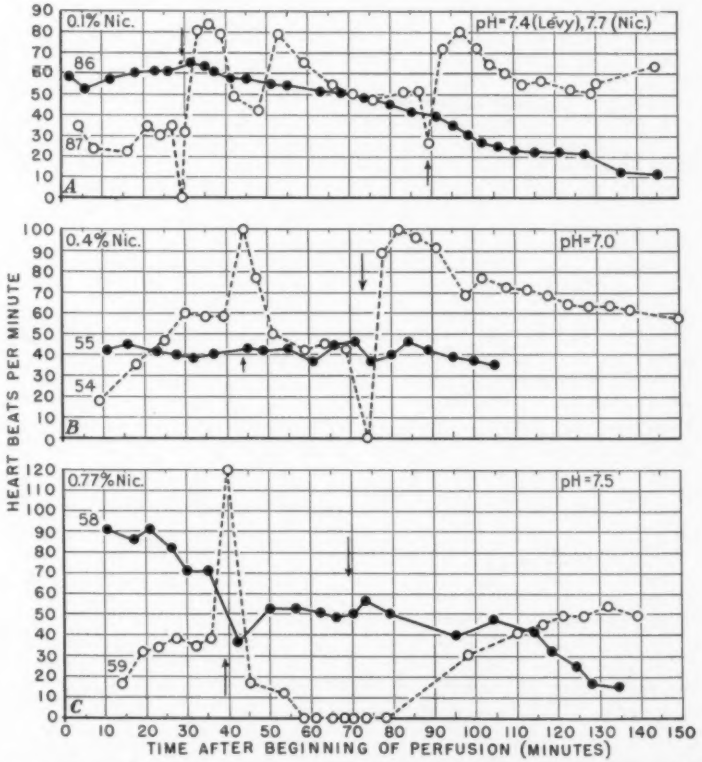

FIGURE 4.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the strip-transfer method to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Continuation of figure 3.

3 and 4, *A* and *B*) or showed a momentary depression of rate at the time of nicotine addition (fig. 2, *D*, no. 176). In all the transfer experiments the inhibitory effects of nicotine on larval heart rate were found to be reversible in the sense that some recovery, as evidenced by an increase in contraction rate, was observed to follow upon the second transfer of heart preparation from nicotine-saline to original saline solution (figs. 4, *C*, and 5).

The contraction rate of the roach heart was inhibited by nicotine at considerably lower concentrations than was that of the larval heart (figs. 1, 4, *C*, and 5, *A* and *B*). In general, when both a stimulation and a depression of contraction rate were detected in a given experiment, the depression, as in the larval heart preparation, occurred subsequent to the initial temporary stimulation, appeared as a more or less rapid decrease in rate to or toward the zero level, depending on the nicotine concentration used, and was reversible in the sense that some degree of recovery followed the transfer from nicotine to original saline (figs. 1, *G* and *H*, 4, *B* and *C*, and 5).

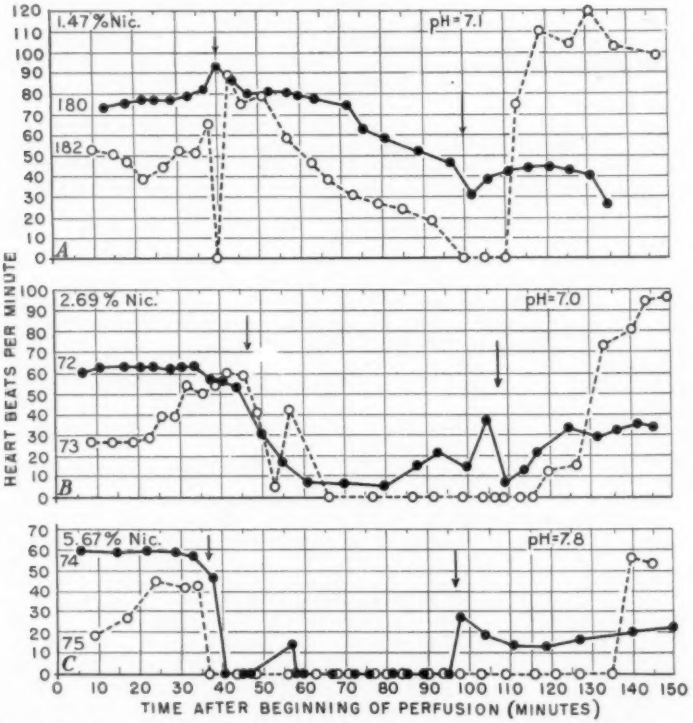

FIGURE 5.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the strip-transfer method to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Continuation of figure 4.

The initial depression of the roach heart contraction rate observed in some of the nicotine concentrations (figs. 3, *F*, 4, *A*, and 5, *A*) when the strip-transfer procedure was used is not considered to be true nicotine depression, since it was also obtained in the control experiments (fig. 3, *A*, *B*, and *C*), although its magnitude may have been influenced by the nicotine. Similar initial depressions have not as a rule been obtained by the addition procedure (fig. 1). In certain nicotine concentrations marked depressions of rate were frequently observed immediately after the second transfer from nicotine to

original saline, prior to the higher recovery level that resulted from the transfer (fig. 4, *A* and *B*).

EFFECTS ON CARDIAC AMPLITUDE

In general, in the case of the larval heart, no decrease of cardiac amplitude appeared to accompany the inhibitory effects of the higher nicotine concentrations. Although a slight increase of amplitude was thought to occur, it was not detected with certainty. Whenever nicotine was observed to reduce the larval cardiac rate to zero, the heart was observed to be in a state of apparent diastolic standstill; at no time in these experiments has nicotine been observed to produce complete systolic arrest of the larval heart.[4]

In the case of the roach heart, amplitude effects of quite a different order were observed to follow the application of nicotine. In those concentrations that gave marked initial stimulation of contraction rate, the quickened rhythm was usually accompanied by an obvious decrease of amplitude in the direction of incomplete relaxation of the cardiac walls, with the result that the rapidly beating heart remained more in systole than in diastole. There was also usually some degree of cardiac incoordination which gave to the heart walls, as they contracted, an appearance of writhing or fibrillation. This fibrillation-like activity was observed to a less marked extent, and as a more transitory effect, in the lower nicotine concentrations, which produced only a slight initial acceleration of contraction rate. Whenever nicotine reduced the roach heart contraction rate to zero within a short time, immediately after cardiac arrest the heart was nearly always found to be in a state of systolic standstill, tightly constricted. Upon the heart preparation remaining in the nicotine solution, however, the cardiac diameter often increased slowly until the heart was neither fully dilated nor fully constricted. Nicotine concentrations too low to cause obvious increase of contraction rate or marked alterations of amplitude seemed, however, to increase the intensity or vigor of contraction.

MISCELLANEOUS OBSERVATIONS

In these experiments it has been noticed that in general the roach heart exhibited somewhat larger and more rapid fluctuations in contraction rate and amplitude than did the larval heart, even before the application of nicotine (figs. 3, 4, and 5).

The contraction rate of the larval heart tended to exceed that of the cockroach heart when the preparations were perfused with nicotine-free saline; in these experiments the average rate of the larval heart was about 58 beats per minute while that of the roach heart was about 49 beats per minute. The rates, however, varied greatly from one insect to another.

Occasionally a condition of heart block was observed in roach or larval heart preparation, more frequently in the latter. The larval heart block occurred sometimes after prolonged perfusion and sometimes with nicotine application. It appeared as one contraction of that portion of the heart lying anterior to the gonads occurring at each second, third, or higher beat of that part lying posterior to the

[4] A larval heart preparation placed in 20-percent nicotine in saline was immediately brought to marked apparent diastolic arrest.

gonads, especially that portion in the rectal segments. In one or two cases the heart block disappeared with cardiac recovery.

In both the roach and larval heart preparations reversal of the direction of cardiac peristalsis was frequently observed. Often the reversals were periodic and occurred fairly rapidly; one roach heart exhibited three or four postero-anterior beats, then three or four anteroposterior beats, and so on, for about 2 hours of observation. Some hearts showed either a postero-anterior or an anteroposterior beat throughout a single experiment, without observed reversal. Between these two extremes occurred varying degrees of reversal periodicity, whether the hearts were in nicotine solution or not. In general, reversals seemed to occur more often in roach than in larval preparations.

That portion of the larval heart lying in the rectal segments usually appeared to be bordered by larger laterally lying masses of tissue than bordered the more anterior regions of the heart. The lateral tissues themselves, in many preparations, exhibited vigorous rhythmic movements, indicating the presence of contained or associated rhythmically contracting muscle fibers. Sometimes the rhythmic activity appeared to be synchronous with the heart contractions, but at other times it was definitely asynchronous. This apparent extracardial rhythm often so interfered with the cardiac contractions, even when their rate continued unchanged, that contraction rates could not be observed in the rectal region but were obtained from the gonadal region. The extracardial activity consisted in the laterally lying tissues being so pulled away from the centrally located cardiac tube as to seem to stretch the latter and interfere with or obscure its contraction.

In many of these experiments diastolic pauses have been observed, usually either during nicotine application or during recovery from nicotine. In many cases apparent irregularities of rate have been caused by occasional diastolic pauses which lowered the average values of the contraction rate at various points on the rate-time curves. Usually when pauses of the nonnicotinized heart occurred, they were diastolic rather than systolic. Systolic pauses were observed frequently in the strongly nicotinized roach heart, sometimes in the nonnicotinized roach heart, but almost never in the larval heart.

Structures that have a superficial resemblance to tubular vessels or channels have been noticed incidentally in the cockroach heart preparations. They extend laterally and at right angles from the region of the heart, especially in the posterior part of the abdomen. During the cardiac cycle they were seen to exhibit rhythmic changes of diameter synchronously or asynchronously with the heart beat. the increase of diameter usually appearing as a more vigorous movement than the decrease of diameter. These apparent vessels or channels occur in pairs, the members of a pair lying on opposite sides of the heart. One member sometimes showed marked rhythmic activity while the other member remained quiescent, though usually both were active at the same time; the activity was persistent or appeared and disappeared several times in a single experiment. Whether these structures are closed tubes or merely open channels has not been determined in this investigation, although movement of liquid in their apparent interior has been observed to accompany their activity. These structures appeared to be the same as the struc-

tures described by Alexandrowicz (*1*) as segmental blood vessels of the cockroach heart.

DISCUSSION

From the results obtained in these experiments it is apparent that, under the conditions described, the cardiac mechanism of the cockroach is considerably more responsive to the action of nicotine than is that of the southern armyworm. The rhythmicity of the latter is stimulated either relatively slightly or not at all and is markedly depressed only by the higher concentrations of nicotine used, whereas the rhythmicity of the roach heart mechanism is stimulated by a wider range of nicotine concentrations and is both initially stimulated and subsequently depressed by the lower concentrations. The roach heart stops in systole and the larval heart stops in partial or complete apparent diastole when arrested by nicotine, and in a given nicotine concentration cardiac arrest occurs earlier in the roach than in the larval heart mechanism. Removal of the preparations back to the original saline solution by a second transfer usually results in an earlier recovery of activity by the larval heart than by the roach heart. The initial rate increase is usually much greater in the roach than in the larval heart preparation. The nicotine-stimulated roach heart often exhibits a fibrillationlike activity that has not been observed in the nicotinized larval heart. Both the rate-stimulatory and the rate-inhibitory effects are essentially reversible processes within the nicotine-concentration range employed in these experiments.

The stimulatory and inhibitory effects of nicotine upon vertebrate and invertebrate hearts have been observed by a number of investigators. One of the more suggestive investigations, from the present standpoint, is that of Carlson (*5*), who studied the effects of nicotine applied separately to the ganglionic and the muscular tissues of the *Limulus* heart. Bringing into consideration other results reported by various authors, he concluded that nicotine affects differently the nervous and muscular tissues not only of the *Limulus* heart, in which anatomical separation of nervous and muscular portions can be readily accomplished, but also of vertebrate and invertebrate hearts generally, in which nervous and muscular elements are not so readily separable. Carlson found that application of nicotine solutions in sea water or plasma to the *Limulus* cardiac ganglia caused a primary (initial) stimulation, exhibited as increased contraction intensity, contraction rate, or both, followed by irregularities of rhythm, depression, or paralysis, depending on the nicotine concentration used; whereas direct application of nicotine solution to the heart muscle had only depressive or inhibitory effects without appreciable associated stimulation, relatively dilute nicotine solutions having no apparent effect. Nicotine concentrations too low to affect the cardiac muscle directly produced marked stimulation when applied to the ganglia. Carlson (*6*) concluded that the action of nicotine upon the vertebrate and the invertebrate heart is primarily upon its intrinsic ganglionic system, and not upon its cardiac muscle or its motor nerve fibers. Carlson's results on *Limulus* bear a suggestive resemblance to the results obtained in the experiments reported in this paper, for the effects of applying certain nicotine concentrations to the *Limulus* heart ganglia and to the cockroach heart preparation are, in both cases, stimulation only (relatively low concentrations) or stimulation followed by more or less rate irregularity, depression, or paralysis

(relatively high concentrations); similarly, application of relatively high nicotine concentrations to the *Limulus* heart muscle and to the southern armyworm heart preparation causes depression or paralysis associated with relatively slight or no stimulation. That a sufficiently high concentration of nicotine can produce stimulation of some striated cardiac or skeletal muscles is indicated by the reports of several investigators (*2*, *3*, *7*, *29*, *30*). The earlier investigations of Langley and Dickinson (*23*) and of Langley (*21*) on the effects of nicotine solutions upon autonomic ganglia in the vertebrate indicate that nicotine applied to the ganglia acts primarily upon the ganglionic cells, which become initially stimulated before the onset of depression.

The question naturally arises, Are the differences in effect of nicotine on cockroach and larval heart preparations caused by differences in their intrinsic nervous mechanism? Little research has been reported concerning the intrinsic nervous mechanism of the insect heart. Alexandrowicz (*1*) studied histologically the nervous mechanism of the cockroach ((*Periplaneta*) *Blatta orientalis* L.) heart and reported the presence of ganglionic cells in the lateral heart nerves (nervi cardiaci laterales). Zawarzin (*43*) investigated the innervation of the heart of the *Aeschna* larva and described intrinsic nerve fibers, but found no intrinsic ganglionic cells. Although Dogiel (*12*) reported the presence of what he considered to be Wagner's "birnförmige Körper, oder Nervenzellen" in the cardiac mechanism of the *Corethra* larva, he failed to find nerve fibers extending from them and presented inconclusive evidence of their nervous nature; Walling (*37*) considered these cells to be nonneural in character. Kuwana (*20*) found no ganglia and no ganglionic cells in the heart of the silkworm (*Bombyx mori*). Maloeuf (*28*) failed to detect ganglionic cells in the lateral heart nerves of the dragonfly larva (*Anax junius* Drury). Thus the literature that we do have on the subject would seem to indicate that the hearts of some insects possess intrinsic ganglionic nerve cells whereas those of others probably do not.[5]

The effects of nicotine injected into the vertebrate or applied to the exposed or perfused vertebrate heart are complicated by the possibility of the nicotine's acting upon the extrinsic sympathetic or parasympathetic (vagal) cardioregulatory mechanisms, the intrinsic ganglionic cells, postganglionic fibers, or autonomic motor endings, the auricular-ventricular bundle (Purkinje fibers), the cardiac muscle fibers, or various combinations of these. It is not surprising, therefore, that various authors have obtained results that are seemingly not in accord as regards the cardiac effects of nicotine. It has been indicated that nicotine may influence heart action by affecting the vagal cardio-inhibitory center (*18*), the intracardiac ganglia (*8*, *19*), the endings of the motor fibers (*29*, *35*), and the cardiac muscles (*5*, *6*, *29*, *30*). A similar complexity is no doubt involved in the effects of nicotine on the insect or other invertebrate heart that is controlled by extrinsic and intrinsic nervous mechanisms.

[5] It is of incidental interest in this connection that Steiner (*35*) and Fries (*14*), applying the method of critical thermal-increment analysis, reported indirect evidence that the heart beats of the cockroaches *Periplaneta americana* and *Blatta orientalis* are under neural control. Garrey (*15*) reached a similar conclusion concerning the *Limulus* heart from a study of the temperature coefficients of whole heart and separated cardiac ganglia. Crozier and Federighi (*11*) arrived at a similar conclusion regarding the heart beat of the silkworm. Even though the thermal-increment analysis method may yield indirect evidence that the respective cardiac activities are under control of nervous tissue, it alone (i. e., in the absence of additional experimental investigation) does not indicate the locus of such neural control. This applies especially to the experiments of Fries (*14*) and Crozier and Federighi (*11*). Steiner (*35*), who used additional experimenta procedures, suggested that the ganglionic cells of the roach's lateral heart nerves constitute the source o neural control of its cardiac activity.

With these observations in mind, it is obvious that a complete explanation of the results reported here is not readily attainable. Nevertheless, it seems probable that with further acquisition of knowledge of the insect heart these results may receive a reasonable explanation in one of the following ways:

(1) The hypothesis can be formed that, although in the cockroach heart preparation the applied nicotine acts upon a cardiac mechanism possessing intrinsic ganglionic cells, in the larval heart preparation it acts on a mechanism lacking ganglionic cells. In accordance with this explanation, the nicotine would affect primarily the ganglionic cells of the roach heart with initial stimulation and subsequent depression and in the larval heart would act primarily upon the intrinsic nerve fibers, their motor endings, or the cardiac musculature, all of which, in the vertebrate, have been shown (*5*, *6*, *21*, *22*, *23*, *24*, *31*, *33*) to be less readily affected, especially in an excitatory manner, by nicotine than are ganglionic cells.

(2) It may be supposed that both heart mechanisms possess intrinsic ganglionic cells but that these cells, the cardiac muscles, and the intrinsic nerve fibers and nerve endings have relatively different resistances to the action of nicotine.

These two possible explanations are presented as working hypotheses, with the view that additional anatomical knowledge regarding the existence of intrinsic ganglionic cells in the two cardiac mechanisms should offer evidence tending to confirm or refute the one or the other.

The effects of nicotine solutions on cardiac amplitude in the roach heart preparation are in general accord with those reported by other investigators who have used hearts possessing intrinsic ganglionic cells. Clerc and Pezzi (*10*) report that auricular fibrillation and flutter, as well as other abnormal cardiac phenomena, can be detected in the dog heart following the injection of nicotine; the fibrillation is attributed (*9*) to the action of nicotine on vagal innervation. Levy (*25*) observed ventricular fibrillation of the anesthetized cat heart subsequent to nicotine injection. Burridge (*3*) found that perfusion of the frog heart with a sufficiently high concentration of nicotine caused cardiac contracture with loss of relaxation capacity. Barry (*2*) observed increased intensity of contractions, appearance of extrasystoles, shortening of the auricular-ventricular interval, and double auricular rhythms among the effects produced by nicotine on the perfused frog heart. The investigations of Langley (*22*) show that nicotine contraction and nicotine contracture [6] are producible in muscle fibers (skeletal), and that the regions of the neural endings are more sensitive to the drug than are the nonneural regions. Although the investigations of these and other authors indicate that nicotine can increase the degree of skeletal and cardiac muscular contraction either by direct action on the muscle fibers or by indirect action via their nerve supply, or both, the mode of action of nicotine in producing the fibrillationlike activity and systolic arrest of the roach heart is not known. It is probable that both an indirect neural and a direct muscular action occur as the concentration of nicotine in the cardiac tis-

[6] "Contracture" is a poorly defined term for an incomplete, reversible, nonpropagated mechanical shortening of muscle not necessarily accompanied by the propagated action current typical of contraction. "Contraction" refers to the usual twitch and tetanus type of muscular response, well known in muscle physiology. Often contracture is used with the meaning of prolonged shortening.

sues is built up, and that the final systolic arrest of the roach heart indicates a direct action of nicotine on the cardiac muscle fibers.

The role of the alary muscles in the insect cardiac cycle and in the cardiac effects of nicotine is not clearly understood. Suggestions have been made that the alary muscles actively participate in diastole (*38, pp. 458, 460*) and that they more or less passively affect the dilation of the heart by slight changes in tension (*44*). Mechanically, insofar as the alary and cardiac muscle tensions are concerned, the insect heart, arrested by nicotine or any other cause, should stop in systole or diastole depending, respectively, upon the less or greater tension of the alary than of the cardiac muscle fibers at the time of cardiac standstill. The present observations indicate that at the time of nicotine arrest the relative tension of the roach cardiac muscle becomes increased (nicotine contracture?). If the cardiac muscle tension of the nicotine-arrested larval heart is also increased, then, since the larval heart does not stop in apparent systole, the tension of the alary muscles must be increased to a relatively greater extent. Federighi (*13*) has found that the peristaltically rythmic dorsal vessel of the annelid *Nereis virens* Sars possesses smooth muscle fibers and is brought to diastolic arrest by nicotine. Zilwa (*44*) has also reported results indicating that nicotine can directly or indirectly decrease the tension of smooth muscle. Barry (*2*) found that the auricles of the perfused frog heart are brought to diastolic standstill by nicotine, although the ventricle of the same heart passes into systolic arrest. The failure of nicotine, even in very high concentration, to produce systolic arrest of the larval heart would seem to indicate either that the cardiac muscle of the southern armyworm is different not only from that of the roach used here but also from the cardiac and skeletal muscle known to exhibit nicotine contracture, or that the larval alary muscles are thrown into contracture of intensity sufficient to overcome that of the cardiac muscle and thereby to produce apparent diastolic standstill.

The initial stimulating action of the lower nicotine concentrations is readily detected by the nicotine-addition procedure, but when the transfer procedure is used this action is partially or entirely masked by the inhibitory effect of the transfer, which occurs in the control experiments, especially in the case of roach heart preparation. This transfer depression of rhythmicity probably results from the mechanical stimulation of the heart mechanism.

The pH values of the saline and nicotine solutions used for perfusion were kept within the approximate range of 6.8 to 7.8, and in a given experiment were equalized or nearly equalized. At one end of this range (pH 6.8) the ratio of undissociated (alkaloid) nicotine (Nic HOH) molecules to the dissociated nicotine ions (Nic H^+) attains the theoretical value of approximately 1:2.4, and at the other end (pH 7.8) the ratio is about 1:63. These values are of the applied solutions, and not of the solutions after penetration into the cardiac tissues where their pH values may become subject to alteration by tissue buffers, especially in the early stages of perfusion; nor is account taken of the nicotine salt (hydrochloride) that is formed in the nicotine-saline solution as a result of the presence of ionizable electrolytes. In any case the dissolved nicotine may be considered to be from about 40 to about 1.6 percent in un-ionized form, while presumably a considerable portion of the ionized nicotine becomes the hydro-

chloride salt. It is to be expected that these conditions would necessitate the use of relatively higher total concentrations of nicotine to affect the cardiac tissues than if the nicotine had been used in unneutralized solution, since several investigations (*6*, *23*, *27*, *32*) indicate that nicotine is more effective when applied to tissues in alkaloid than in salt form.

It has been observed incidentally in these experiments that, if certain nicotine concentrations that do not arrest the perfused larval hearts are injected into intact larvae, the larvae can be quickly and completely paralyzed, although their intact hearts continue to pulsate. It would appear, therefore, that the cardiac tissues of these insects, especially the southern armyworms, possess higher resistance to the action of nicotine than do the intact insects. Shafer (*34*) noted that cardiac activity was one of the last visible indications of life in insects killed with various poisons, and Walling (*36*) observed that various gases decreased cardiac activity of the grasshopper less readily than respiratory activity.

The causes of the large nonnicotine fluctuations in rate shown by most of the rate-time curves, including those from the control experiments, are not known. They have occurred throughout these experiments and can be observed in contraction rate-time curves of intact insect hearts presented by other investigators (*4*, *39*). Thus, both the isolated and the intact insect hearts exhibit variations of this sort, at least when measured by stop watch. It should be emphasized that the contraction-rate values obtained in these experiments and in the experiments of many other investigators are average values representing given contraction periods, and therefore give no indication of the variability of the individual beats that lie within those periods and tend to give inexact representations of some cardiac phenomena, such as extrasystoles and dropped beats. Stop-watch measurements of contraction rate yield only a portion of the desired information concerning the effects of nicotine on the insect (or other) heart. It is known that nicotine acting on the noninsect heart can produce changes in amplitude as well as in rate. This has appeared to be true in some of the present experiments in which low concentrations of nicotine seem to increase cardiac vigor. In the experiments reported here, therefore, only that portion of the nicotine action upon the roach and larval hearts which is exhibited as alteration of contraction rate has been measured.

The causes of reversal in the isolated heart preparations are not known. Yokoyama (*42*) has presented evidence that reversal in the silkworm heart is caused, partly at least, by factors such as oxygen supply, temperature changes, or applied adrenalin, which produce increases or decreases of automatic rhythmicity at one end of the heart relative to the other end. Gerould (*17*) has also produced cardiac reversals by altering the oxygen supply to the intact insect heart, but he has also observed (*16*) reversals in the isolated heart preparation. It seems doubtful that variations in oxygen supply caused the reversals observed in these experiments, since reversals occurred under conditions of continuous aeration of the perfusion fluids; nor, since reversals occurred in nicotine-free saline, are they entirely attributable to the alkaloid. It is of interest, nevertheless, that Barry (*2*) obtained ventricular-auricular rhythms, as well as other coordination disturbances, in the nicotine-perfused frog heart.

SUMMARY AND CONCLUSIONS

Isolated heart preparations from the fifth- or sixth-instar southern armyworm (*Prodenia eridania* Cram.) and the American cockroach (*Periplaneta americana* L.) have been perfused with various concentrations of nicotine to determine the effect on cardiac contraction rate. Two methods of perfusion have been used, the strip-transfer procedure and the nicotine-addition procedure, and the results and conclusions drawn apply only to the above-named insects and under the specified conditions of nicotine application.

The transfer procedure does and the addition procedure does not result in a frequently occurring initial cardiac depression of rate, which is considered to be nonnicotinic in origin.

In general, with regard to contraction rate, nicotine applied to the roach and larval heart preparations causes initial stimulation without depression at relatively low concentrations, initial stimulation followed by partial depression at relatively intermediate concentrations, and stimulation followed by complete depression and paralysis at relatively high concentrations.

The magnitude of the effect of nicotine upon contraction rate is in general much greater in the roach than in the larval heart preparation.

The stimulatory and inhibitory responses of the cardiac mechanism are elicited by relatively lower nicotine concentrations applied to the roach than to the larval heart preparations.

Both the stimulatory and the inhibitory effects are essentially reversible.

The nicotine-arrested roach heart is brought to systolic standstill, whereas the nicotine-arrested larval heart is brought to partial or complete apparent diastolic standstill; in general, the pulsating roach heart does and the pulsating larval heart does not tend toward greater apparent systole under the action of nicotine.

The roach heart can be stimulated by nicotine to exhibit a fibrillationlike activity that has not been observed to result from similar stimulation of the larval heart.

The roach and larval hearts may exhibit considerable fluctuation of contraction rate whether under the influence of nicotine or not; the fluctuations tend to be more marked in the roach than in the larval heart.

The roach and larval hearts may exhibit reversals of direction of pulsation (reversals of beat) whether or not they are under the influence of nicotine.

It is suggested that the marked differences in response of the roach and larval isolated cardiac mechanisms to perfusing nicotine may be due to differences either in their possession of intrinsic ganglionic cells or in the resistance to nicotine of various of their component neural or muscular parts.

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THE BEET LEAFHOPPER IN THE CENTRAL COLUMBIA RIVER BREEDING AREA [1]

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INTRODUCTION

The Yakima Valley in Washington is a potential sugar-beet-producing area, but production is limited by the prevalence of curly top disease, caused by the beet leafhopper, *Eutettix tenellus* (Baker). Three sugar factories built before the severity of this disease was realized have now been dismantled. An attempt to produce white pea beans commercially in the vicinity of Walla Walla in 1930 was abandoned, apparently for the same reason, and tomato growers there suffer heavy losses almost every year. In fact, susceptible crops in vegetable gardens throughout this section are severely damaged.

Surveys conducted by the Bureau of Entomology from 1927 to 1929 [3] indicated that there is a large breeding area of the leafhopper in the central Columbia River Valley, in south-central Washington and northeastern Oregon, from which populations are dispersed to the Yakima Valley and other surrounding territory (fig. 1).[4]

In 1930 ecological studies of the beet leafhopper were undertaken in this area. A laboratory was established at Hermiston, Oreg., and 24 stations were set up for the purpose of making observations on this insect. These stations were so placed as to include the more important host plants and were widely enough separated to be representative of the entire breeding area. To determine the host-plant sequence, brood occurrence, and population fluctuations throughout the season, observations were made at these stations as nearly as possible every 10 days from spring to fall over the period 1930 to 1933. Population sampling was accomplished the first year by means of the sweep net, supplemented by square-yard counts in the spring and late in the fall, when the temperature was so low and host plants were so small as to make sweeping impracticable. The square-yard counts were made by covering a definite area on hand and knee and counting the insects disturbed. The square-foot sampler,[5] a cylindrical cage with a 5-foot handle and covering 1 square foot of soil surface, was devised in the spring of 1931, and was used exclusively after that time.

LIFE HISTORY

The beet leafhopper overwinters in the central Columbia area only as the fertilized female. During the year there are at least three broods. The spring brood appears in May, the summer brood about

[1] Received for publication Mar. 1, 1937; issued August 1937.

[2] The writer's thanks are due P. N. Annaud, formerly in charge of this project, for guidance and suggestions in the study of this problem; and to W. C. Cook for helpful suggestions in the preparation of the manuscript.

[3] These surveys were conducted by H. E. Wallace, C. H. Griffith, and the writer.

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the first of July, and the fall brood late in August (fig. 2). The spring and summer broods overlap, causing the peak of populations for the

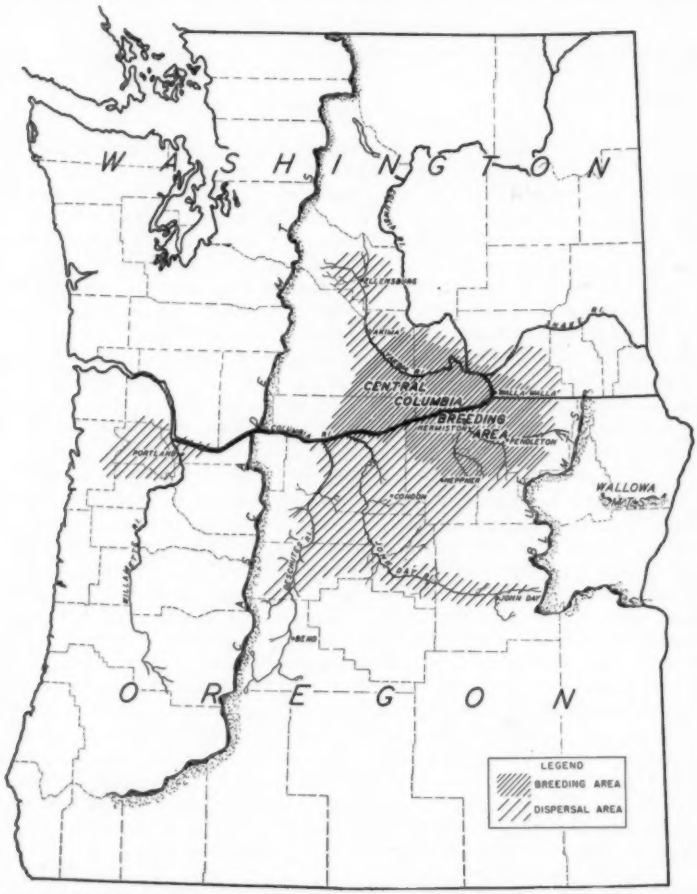

FIGURE 1.—Central Columbia River breeding area and area reached by dispersal from this breeding ground.

season to come in July or August. There is also an overlapping of the summer and fall broods, but host-plant and weather conditions

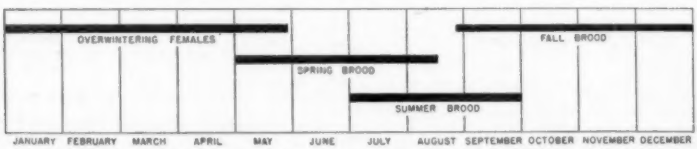
OVERWINTERING FEMALES
SPRING BROOD
SUMMER BROOD
FALL BROOD
JANUARY FEBRUARY MARCH APRIL MAY JUNE JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER

FIGURE 2.—Occurrence of the different broods of the beet leafhopper in the central Columbia breeding area.

at this time are such that populations are not so high as during the summer months.

HOST PLANTS

The principal overwintering host plants of *Eutettix tenellus* in this area are alfileria (*Erodium cicutarium*) and tumblemustard (*Norta altissima*). This mustard is also the most important host of the spring brood. Russian-thistle (*Salsola pestifer*) is by far the most important host of the summer brood. The fall brood matures on Russian-thistle, but the drying of this plant forces the insects to shift to the young fall-germinated plants of mustard and alfileria.

HOST OF THE SPRING BROOD

Spring-brood nymphs hatch and develop largely on tumblemustard and alfileria. These plants germinate in the fall and are usually abundant early in the spring. The tumblemustard usually lives longer than the alfileria, but since the two occur in close proximity or in intermixed stands the insects easily shift from alfileria to tumblemustard. Ordinarily neither plant dries before most of the spring brood matures, and many of the eggs are deposited in the mustard. Each year summer nymphs have hatched from the mustard just as it dried.

Tumblemustard is subject to considerable competition by downy bromegrass (*Bromus tectorum*). Much of the area in which tumblemustard was dominant in 1930 was entirely replaced by bromegrass in 1933. Alfileria is not so easily crowded out by the bromegrass, since it grows much faster late in the fall and early in the spring than does either tumblemustard or bromegrass. Moisture is sufficient for both plants at these times, and alfileria usually matures by the time the bromegrass becomes large enough to crowd it out. The tumblemustard, maturing later, comes into direct competition with the bromegrass at a time when moisture is scarce.

Large acreages of tumblemustard were noted to have succumbed to drought since 1929. In the summer of 1929 this mustard was abundant over much of the area between the Columbia and the Yakima Rivers. In 1930 young plants were observed the middle of March, but they had dried by the middle of April. Very few plants were seen in 1931, and most of these dried before reaching maturity. As a result the area was almost barren of tumblemustard during 1932 and 1933.

In 1932 *Pectocarya penicillata* harbored large populations of spring-brood nymphs and adults. Previously the plant had not been noticed. Its distribution was limited, and it was found to be relatively short-lived. In 1932 its seeds germinated late in February and the plants were mature and dry by May; in 1933 the seeds germinated the first week in March and the plants had matured and dried by the first week in June. In most places many large nymphs of the spring brood were present at the time the plants dried.

Bassia hirsuta, though of limited distribution, harbors rather large populations of the spring brood. It germinates either in the fall or spring and remains green until the following fall. Germination was noted on October 27, 1930, November 18, 1932, March 7, 1931, and March 14, 1933.

Chenopodium leptophyllum is a host of minor importance. Prior to 1933 population counts had not been made on this plant owing to its limited distribution. In 1933 it was slightly more abundant, but spring-brood populations on it were comparatively small.

HOSTS OF THE SUMMER BROOD

Russian-thistle, the most important summer host, has germinated in March every year, but it has not harbored large populations until after the spring dispersal. The entire life cycle of the summer brood is spent on this plant.

Gilia minutiflora is primarily a host of the summer and fall broods. The seeds usually germinate in April and the plants do not dry until late in October. Where *Gilia* is intermixed with tumblemustard, as it often is, populations on it are small until the mustard becomes unsuitable; where it occurs away from spring hosts, populations prior to dispersal are small. Populations have never been known to be large on this host, but in some years its abundance has made it important. In 1930, when it first attracted attention as a host of *Eutettix tenellus*, it was very abundant. That summer it was completely defoliated by the larvae of *Heliothis* sp. Later the plants leaved out and bloomed to a limited degree, but before they could set seed they were again defoliated, this time by a second brood of *Heliothis*. The plants were so completely prevented from setting seed by these two broods of *Heliothis* that *Gilia* was not again abundant until 1933, and even then it was less so than in 1930.

Chenopodium leptophyllum is a minor host of the summer as well as of the spring brood, since it does not usually dry until September.

HOSTS OF THE FALL BROOD

Host plants of the fall brood are of three distinct types: (1) Those on which the brood hatch and develop, (2) those on which the females overwinter, and (3) those that furnish food and a medium for oviposition the following spring. Of the first group Russian-thistle is by far the most important, with some breeding occurring on *Gilia minutiflora*, *Chenopodium leptophyllum*, and *Bassia hirsuta*. Alfileria is the most important in the second group owing to its abundance, while tumblemustard is the most important in the third group. This mustard germinates early in March and large spring populations of nymphs indicate that it is preferred for oviposition by the overwintered females.

SEASONAL OCCURRENCE

The magnitude of the spring brood of *Eutettix tenellus* depends upon the numbers of overwintered gravid females present in the spring. Thus, fall drought, type of winter, or any other factor that affects the overwintering of the fall-brood females has a marked effect on spring-brood populations. The time of appearance of this brood depends largely upon the temperature.

As will be seen from figure 3, spring-brood nymphs appeared each year about the first of May [6] and reached the peak of abundance the latter part of May. The low level the middle or latter part of June represents the end of that brood. The curves did not reach zero in June because of the hatching of the summer-brood nymphs and consequent overlapping of broods.

In 1931 an abundance of spring nymphs was expected because of the relatively large populations of overwintered females (fig. 3) and

[6] The data for 1930 were obtained by sweep-net sampling and are in terms of insects per 50 sweeps of a 14-inch net. They are not comparable to those obtained from the square-foot samples and are therefore not presented in figure 3.

the early egg development (fig. 6). Such is not indicated in the curve for that year. This inconsistency was probably due to imperfections in the technique of removing and counting small nymphs, since it was the first spring the counting cage was used. Sweep-net samples and the early appearance of large numbers of spring-brood adults indicated that nymph populations were comparatively large during May 1931.

In 1930 and 1931 spring-brood adults appeared from the middle to the latter part of May, populations reaching their peaks in June and declining after July 1, but in 1932 and 1933 they did not appear until about June 1, reaching their peaks the last of July. In figure 3 disappearance of the spring-brood adults cannot be determined, since the summer brood appeared about the first of July and caused overlapping.

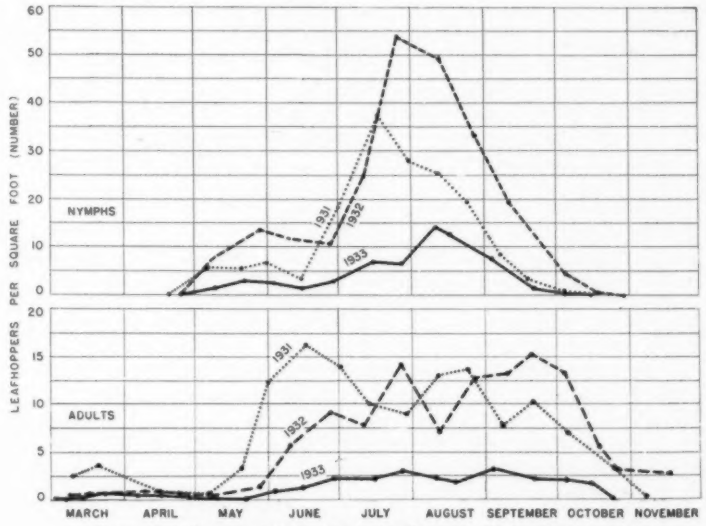

FIGURE 3.—Mean populations of the beet leafhopper in the breeding grounds.

Each year there has been a sudden increase in nymph populations and an appearance of very small nymphs late in June, which probably denotes the hatching of the summer brood. The summer and fall broods greatly overlap, so that figure 3 scarcely shows where one leaves off and the other begins. The slight decrease toward the end of July probably represents the beginning of the decline of the summer-brood nymphs. The time of appearance of the summer-brood adults is masked by the presence of the spring-brood adults. The peak of summer-brood adults came in August in 1930 and 1931, and the first of September in 1933. In all probability the 1932 peak also came the first of September, although this is not shown in figure 3, because the early appearance of large numbers of fall-brood adults undoubtedly prevented a drop in September populations.

The time of hatching of the fall-brood nymphs cannot be determined from figure 3, but it is undoubtedly the hatching of this brood that prevents the curve from dropping to zero in August, as the trend

the latter part of July would indicate. Figure 3 shows a peak in September for fall-brood adults in 1931 and 1932. In 1933 populations were small, and in September fall-brood emergence scarcely replaced summer-brood mortality. Therefore, no fall-brood peak is shown for this year. The definite drop in adult populations in October is caused by the drying of the summer hosts, principally Russian-thistle. The last point in the 1931 curve for adults and the last two points in the 1932 curve represent collections on newly germinated mustard and

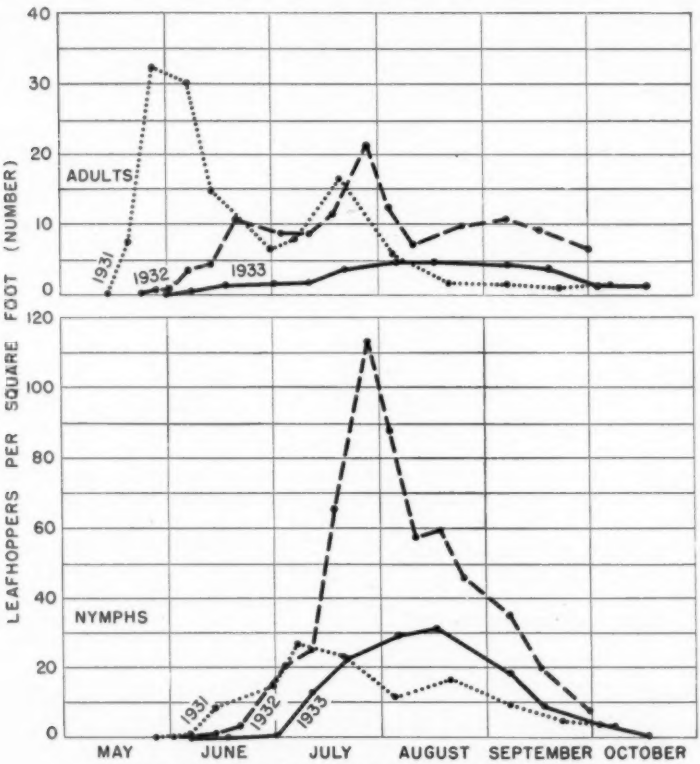

FIGURE 4.—Mean populations of the beet leafhopper in the beet plots.

alfileria. As fall hosts cover a much larger area than summer hosts, the shift results in a decreased population density, but there is probably a real mortality due to the forced shifting. The small fall populations shown are a resultant of both these causes.

DISPERSALS

Movements or dispersals of the spring brood have been studied by observations in beet plots and by catches in insect traps. Since no sugar beets are grown in the central Columbia area, one-fourth-acre plots were planted to beets in cooperation with the farmers for the

purpose of studying *Eutettix tenellus* populations as well as disease conditions. Three were planted in 1931, three in 1932, and four in 1933. Prior to the spring dispersals the beet plots contained only a few overwintered females, which had no doubt come from adjoining wild hosts. Therefore, the increase in adult populations during May and June (fig. 4) was due entirely to an influx from the breeding ground. The curves for adults in figure 4 indicate both the time and the relative magnitude of the dispersals for these years.

A comparison of figures 3 and 4 shows that while adult populations are large in the breeding grounds at the time of migration, the peak is not reached until migration is over. It is possible that migration is so closely associated with brood maturity in the breeding grounds that the peak of adult populations is prevented until after migrations are over.

Approximate dates of spring dispersals as determined by windvane trap [7] collections and beet-plot population studies are as follows:

1931	May 19–20, 25–30. June 1–12. July 6–15 (slight).
1932	May 25 and 31. June 1–25. July 1–5 (slight).
1933	June 1–19 (slight).

In 1931 four traps were placed on the city water tower at Stanfield, Oreg., at heights of 38, 74, 108, and 127 feet above the ground, to determine the relative numbers of insects at those heights. During the season 18 *Eutettix tenellus* were caught in the trap at 38 feet, 12 at 74 feet, 6 at 108 feet, and 10 at 127 feet. Although these data are meager, they indicate that the flying insects are most numerous close to the ground.

The records obtained indicate that large populations in the breeding ground precede heavy dispersals, and since it is the spring brood that disperses to the cultivated area, the magnitude of this brood in the breeding ground is of prime importance. Figure 3 shows that the spring-brood populations in the breeding grounds were much larger in 1931 than in 1932 and 1933. Likewise, figure 4 shows a much larger spring-brood population in the beet plots in 1931 than in either 1932 or 1933. Curly top was severe in the plots in 1931, but not in 1932 and 1933. Curly top injury to tomatoes over the Umatilla irrigation project was also more severe in 1931 than in either 1932 or 1933. The magnitude of the summer brood in the beet plots, as indicated by the July peaks in figure 4, is of comparatively little importance, since the older plants are more resistant to the disease.

FALL AND WINTER SURVIVAL

Survival of the fall brood may be divided into two periods, fall and winter. Fall survival is largely dependent upon proper host-plant sequence. A change of host is necessitated by the maturation of Russian-thistle, which usually occurs from September 15 to October 15. High fall survival is favored when rainfall is sufficient to germinate winter hosts before Russian-thistle matures. Fall survival is low when fall rains are late and germination is delayed until after

[7] FULTON, F. A., and CHAMBERLIN, J. C. A NEW AUTOMATIC INSECT TRAP FOR THE STUDY OF INSECT DISPERSION AND FLIGHT ASSOCIATIONS. Jour. Econ. Ent. 24: 757–761. 1931.

Russian-thistle reaches maturity. When this occurs the insects are forced to feed on unfavorable food plants until winter hosts germinate.

In the period covered by these studies four distinct types of winter were represented. During January of the winter 1929–30 there were 16 days of subzero weather with a minimum temperature of −38° F., accompanied by 15 inches of snow. Snow was on the ground 5 days during December and 19 days during January. During the winter of 1930–31 there was no snow and the minimum temperature was 15°. The winter of 1931–32 set in early. The temperature dropped to 8° on November 21 and was consistently low from then to the middle of December, with a minimum of 2° on November 28. There were 5 inches of snow on the ground during this period. Temperatures during the last half of December and all of January were moderate, but from February 1 to 5 they were below 0°, with a minimum of −20° on February 2. The soil-surface temperature on this date was 26°. These low temperatures were accompanied by 8 inches of snow. From November 25 to February 10 snow was on the ground for 52 days. During the winter of 1932–33 there were 7 days of subzero temperature, with a minimum of −9° in December; there was no snow cover during this period, and the soil-surface temperature dropped to 2°. On February 9 the minimum air temperature was −5° and, with no snow cover, the soil temperature dropped to 5°.

The winter of 1932–33 is the only one of the four which had any apparent effect on leafhopper survival. Field counts taken in fall and spring, as well as hibernation-cage results, show that the winters of 1929–30, 1930–31, and 1931–32 had little if any effect on the insects, whereas only 4 percent of the insects survived the winter of 1932–33. The extremely low soil-surface temperatures affected the insect not only directly but indirectly by limiting the food supply, since practically all alfileria and tumblemustard were killed.

SEASONAL COLORATION

Severin [8] mentions certain differences in color patterns of *Eutettix tenellus* in California. These seasonal color patterns have been found to occur in Oregon. In general there are three color types, the dark forms, which are the usual overwintering type, and the green and straw-colored forms, which occur during the spring and summer. In 1931 and 1932 *E. tenellus* adults taken in the regular population counts were classified according to color (fig. 5). The difference in coloration between the green and the straw-colored forms during the spring and summer is not always clear-cut, but there is always a noticeable difference between the spring and summer forms and the dark overwintering forms. This difference in coloration may or may not be associated directly with brood occurrence, but it will be noted from figure 5 that early in the spring, when only overwintered females are present, only the dark forms occur. The sudden increase in May or June is due entirely to the green forms. The peak of the summer brood is caused largely by the straw-colored forms, and the fall populations are made up almost entirely of straw-colored and dark forms. The fall and winter mortality of the straw-colored forms is very high, so that by spring the populations consist almost entirely of dark forms.

[8] SEVERIN, H. H. P. LIFE-HISTORY OF THE BEET LEAFHOPPER, EUTETTIX TENELLUS (BAKER), IN CALIFORNIA. Calif. Univ. Pubs. Ent. 5 (4): 37–88, illus. 1930.

EGG DEVELOPMENT

A special technique was devised for studying seasonal ovule development. About every 10 days samples of females were taken from a locality in which the host-plant sequence was adequate to maintain the insect throughout the year. These samples were preserved in a 5-percent aqueous solution of chloral hydrate. Twenty-five females for each date of collection were dissected under a binocular microscope, and then measurements were made by substituting a sliding-scale ocular micrometer for one of the oculars. Comparative development was determined by measuring the developing ovules within the female, and egg length was taken as an indication of the degree of development. Only ovules over 0.20 mm in length were recorded, smaller ovules being considered unimportant for this work. Results were expressed in terms of total length of all eggs over 0.20 mm for each female, and the mean total length of eggs for each sample was taken to represent

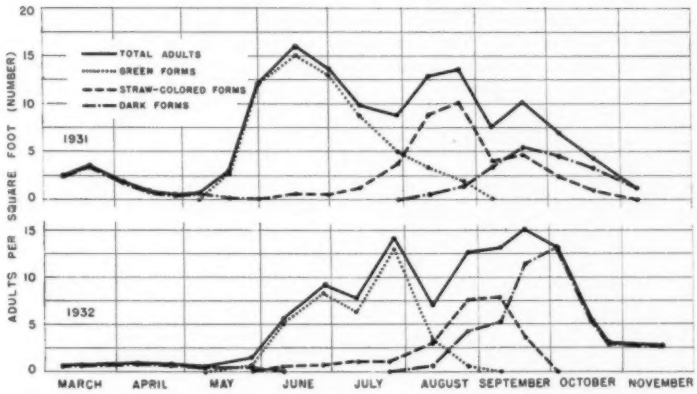

FIGURE 5.—Seasonal coloration of the adult beet leafhopper in the central Columbia breeding area.

the relative egg development for that date. It was later found, however, that almost identical results could be obtained by simply counting the number of mature eggs present in each female on given dates.

The results of these measurements are presented in figure 6. The data indicate a total absence of developing ovules from mid-September until the latter part of February or the first of March of the following year. During April and May the number of developing ovules is much greater than throughout the summer. The decline in May is probably due to exhaustion of the egg supply of the overwintered females and is followed by maturing of spring-brood females.

A peak of ovule development is shown near the end of June 1931 for the spring brood and another the first part of August 1931 for the summer brood. In 1930 a peak is shown for the spring brood the latter part of June. Other peaks for the spring and summer brood do not show in figure 6. They are in all probability masked by overlapping of broods. The abrupt ending of development in September is due

largely to the dying of the summer brood, leaving only the fall females, whose ovaries do not develop until the following spring.

Comparison of figures 6 and 7 indicates that the number of ovules present in the spring females that moved into the beet plots was considerably larger than in those that remained in the breeding grounds.

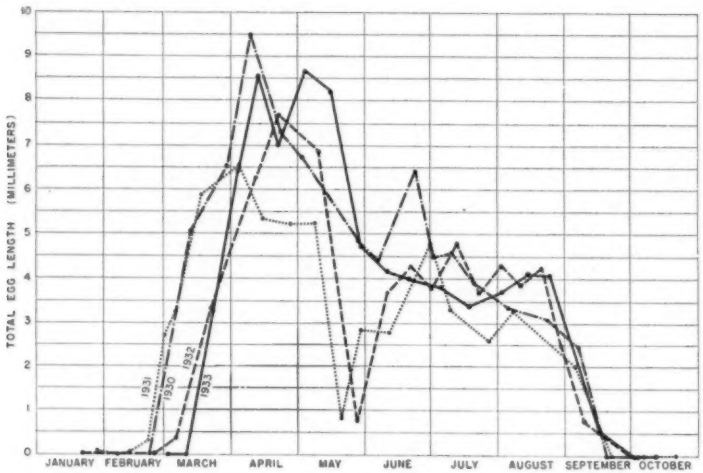

FIGURE 6.—Egg development of the beet leafhopper in the breeding grounds.

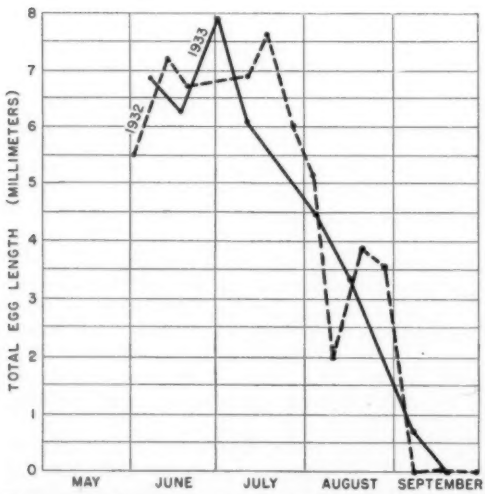

FIGURE 7.—Egg development of the beet leafhopper in the beet plots.

SUMMARY

Ecological studies of the beet leafhopper, *Eutettix tenellus* (Baker), were made in the breeding area in the central Columbia River valley, in south-central Washington and northeastern Oregon. In this area there are three broods of this insect. The more important host plants are tumble-mustard, alfileria, and Russian-thistle.

The leafhopper usually disperses from this breeding area between May 20 and June 30, movements being more or less continuous during this period. Return to the fall and winter hosts, tumblemustard and alfileria, occurs during October and is largely a forced movement caused by the drying of the summer

hosts, principally Russian-thistle. If germination of the fall hosts is delayed by drought until after Russian-thistle has dried, a high mortality is likely to result.

High percentages of curly top are directly correlated with large spring-brood populations of the leafhopper. Large populations of the summer and fall broods are relatively unimportant in this respect, except as they affect the magnitude of the spring brood the following year.

Winter mortality is usually of comparatively little importance in this area. In only one winter of the four observed was there severe mortality, and this was due to subzero weather without snow cover, which not only subjected the insects to intense cold but killed their food plants as well.

Seasonal variations in color patterns of the beet leafhopper have been observed in this area. In general, dark forms predominate during winter, greenish forms during spring, and straw-colored forms during summer.

Studies have been made of seasonal ovule development in this breeding area. Development drops to zero in September and does not begin again until late in February or early in March of the following season.

A TRANSMISSIBLE MOSAIC DISEASE OF CAULIFLOWER [1]

By C. M. TOMPKINS [2]

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INTRODUCTION

A hitherto undescribed mosaic disease of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) was first observed in November 1932, in a field near Alvarado, in the San Francisco Bay section of California. Since cauliflower is a major winter crop in certain coastal valleys in central and southern California, an investigation of the disease seemed desirable. A preliminary report has already been published (*21*).[3] Results of experimental studies on symptomatology, transmission, host range, and identity of the virus are presented in this paper.

REVIEW OF LITERATURE

Within the last 15 years, numerous contributions have been made on virus diseases of cruciferous plants (*1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 16, 18, 19, 20, 23*). These papers, with one exception (*10*) have dealt principally with symptomatology, methods of transmission, and host range, but have presented no data on the properties of the virus or viruses concerned. Hoggan and Johnson (*10*) were the first to report on the properties of a crucifer virus which is transmissible to cabbage, mustard, turnip, and horseradish. In the absence of data, it is doubtful whether the specific identity of other crucifer viruses has been clearly established. When considered as to available facts, all of the crucifer viruses referred to are apparently different from the cauliflower virus reported in this paper.

Only five references to cauliflower mosaic have been found in the literature. Clayton (*3*), in his investigations of "the true cruciferous mosaic", transmitted a virus derived from infected rutabaga plants to healthy cauliflower seedlings. The symptoms consisted of yellow spots scattered over the green leaf, which condition he termed "mosaic." High temperatures (70° to 80° F.) favored symptom expression, while low temperatures (55° to 65°) induced masking. The maximum infection observed in commercial cauliflower plantings was 0.75 percent, and mosaic was stated to be rare. If Clayton's description of symptoms and photographs may be used as criteria for comparative purposes, it is concluded that "the true cruciferous mosaic" is different from the mosaic disease reported in this paper.

[1] Received for publication Nov. 2, 1936; issued August 1937.

[2] The writer gratefully acknowledges his indebtedness to Prof. Ralph E. Smith and Dr. M. W. Gardner for helpful advice and assistance; to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities; to Elizabeth Wilson for seeds of *Brassica arvensis;* to Dr. R. E. Clausen, for seeds of *Nicotiana langsdorffii;* and to Dr. K. Nakata, Kyushu Imperial University, Fukuoka, Japan, for seeds of *B. pekinensis.*

[3] Reference is made by number (italic) to Literature Cited, p. 45.

Noble et al. (*15*) observed a mosaic disease of cauliflower in New South Wales, Australia, in 1933. In a letter to the writer, Noble [4] stated:

> Our cauliflower mosaic is probably distinct from the Californian type. It is not an important disease here, but infected plants are stunted and show mottling and distortion of the foliage. No necrotic spotting has been observed. The disease is thought to be transmitted by the cabbage aphis, but this has not been studied in detail.

Brief reference to a mosaic disease of cauliflower in Japan was made by Hino (*9*).

In 1935 Smith (*19*) observed a mosaic disease of cultivated crucifers in the Cambridge district of England. He found the virus was readily transmissible to various crucifers, including cauliflower, by means of juice inoculations. Initial symptoms consisted of vein clearing, followed by mottling, distortion, and rosetting. No infection of *Nicotiana glutinosa* L. was obtained. These facts suggest a close similarity between the English virus and the cauliflower virus prevalent in California.

Recently, McWhorter [5] in reporting a mosaic disease of dames violet (*Hesperis matronalis* L.), stated that mosaic diseases of crucifers, especially of cabbage and cauliflower, had been observed in past seasons near Oregon City, Oreg. In October 1935, the writer found a mosaic disease of cauliflower in a commercial planting 5 miles north of Salem, Oreg. The symptoms were typical of the disease which occurs on cauliflower in California.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF THE DISEASE

The mosaic disease of cauliflower is coextensive with cauliflower culture in the coastal valleys of central and southern California. Field observations indicate that all commercial varieties of cauliflower grown in the State are liable to infection. The average incidence of the disease seldom exceeds 30 percent of the plant population. Occasionally in a few fields all plants are infected, but such instances are uncommon. From the producer's standpoint, the principal loss caused by the disease is a marked reduction in the number of marketable heads or curds.

Numerous lots of diseased cauliflower plants, representing most of the important commercial varieties grown in California, were collected at intervals during the seasons of 1933 to 1935, inclusive, in the San Francisco Bay section; in the Salinas, Santa Clara, and Santa Maria Valleys; and in Los Angeles County.[6] One collection was also made inland near Merced in the San Joaquin Valley. Proof of natural infection of field-grown plants was established by successful greenhouse inoculations to healthy young cauliflower plants of the corresponding varieties.

Annual surveys made from July to September 1933 to 1935, inclusive, failed to reveal the disease in any commercial cauliflower seedbeds located in the open field, except as noted in the next paragraph. The prevalence of high summer temperatures and the absence of infective aphids may possibly be correlated with lack of infection.

[4] Letter dated Sept. 11, 1935, from R. J. Noble, Department of Agriculture, Sydney, New South Wales, Australia.

[5] McWHORTER, F. P. MOTTLING OR BREAKING IN DAME'S ROCKET IN OREGON. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Reptr. 20: 199. 1936.

[6] Specimens of diseased cauliflower plants were collected near Hawthorne, Los Angeles County, by J. H. Freitag, Division of Entomology and Parasitology, California Agricultural Experiment Station.

In the market-garden areas near Colma, on the San Francisco peninsula, low temperatures prevail throughout the year, permitting continuous cultivation of cruciferous crops, especially cauliflower. Thus a constant source of virus inoculum is available. Infected seedlings are frequently found in seedbeds, usually located at some distance from the cauliflower fields, and the incidence of the disease may vary from a trace to 100 percent.

SYMPTOMS OF THE DISEASE

The sequence of symptom development in cauliflower plants is identical whether the plants are exposed to natural infection in the field or subjected to artificial inoculation in the greenhouse. The first symptom of infection is a clearing of the veins, which usually begins at or near the base of the leaf and gradually extends over the

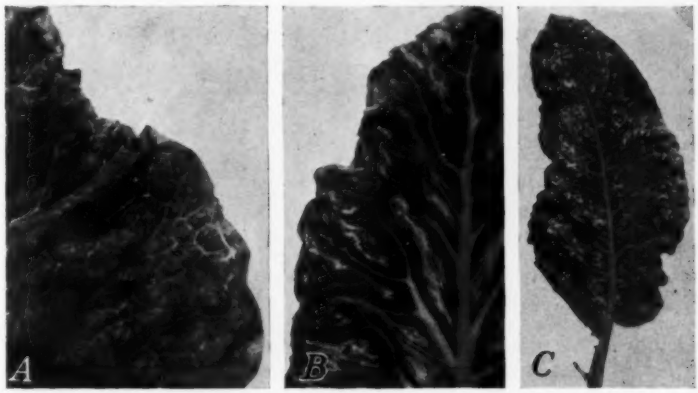

FIGURE 1.—Symptoms produced by the cauliflower mosaic virus on cauliflower leaves under natural conditions: *A*, Vein clearing; *B*, vein banding, mottling, and distortion; *C*, necrotic spotting, curvature of the midrib, and distortion.

entire leaf (fig. 1, *A*; fig. 2). At the onset of the disease, one or several leaves may show vein clearing, but frequently this symptom appears simultaneously on all leaves of the plant.

Vein clearing may persist for 10 to 20 days, but gradually changes to vein banding, which consists of narrow, continuous, dark-green areas parallel with and adjoining the midrib and lateral veins (fig. 1, *B*). The occurrence of numerous, irregularly shaped, nonraised, dark-green islands in the chlorotic areas between the veins results in a mottled effect (fig. 1, *B*).

Later, small, irregular, necrotic lesions appear in the mottled areas (fig. 1, *C*). The lesions, whose light tan color, remains unchanged by age, are different from and cannot be confused with the "ringspot" virus of cabbage and Brussels sprouts recently described by Smith (*19*). A necrotic ring or black-spot virus disease, similar to Smith's ringspot disease but different from cauliflower mosaic, has been under observation and study in the San Francisco Bay section since 1932. The production of necrotic lesions by the cauliflower virus is often accompanied by curvature of the midrib and leaf distortion (fig. 1, *C*).

Early infection usually causes severe stunting of the plants. The terminal heads or curds of stunted plants measure less than 4 inches in diameter, are loosely or not at all covered by small, distorted leaves, and are always slightly discolored and unmarketable. As a rule, late-infected plants are not stunted. Their curds are undesirable for eastern shipment because the mottled leaves often become bleached or yellowed in transit.

Symptom expression in the field depends upon low winter temperatures. Under greenhouse conditions symptoms are well developed on

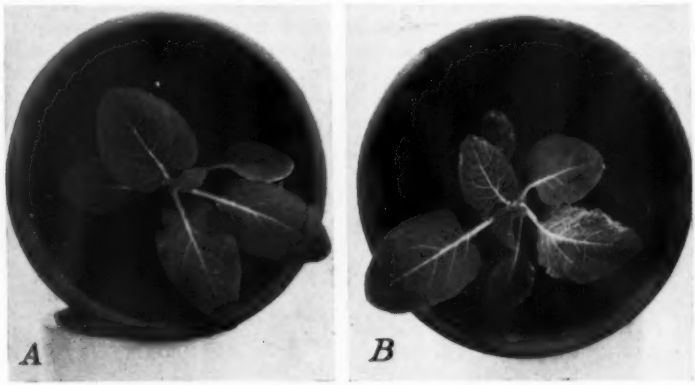

FIGURE 2.—Symptoms produced by the cauliflower mosaic virus on February cauliflower seedlings 15 days after artificial inoculation in the greenhouse at 13° to 19° C.: *A*, Noninoculated control; *B*, infected with the cauliflower mosaic virus.

artificially infected seedlings at temperatures ranging from 10° to 19° C., while higher temperatures favor masking.

MATERIALS AND METHODS

For use in studies on transmission, properties, host range, and varietal susceptibility, a stock culture of the cauliflower mosaic virus was derived in February 1934 from an inner leaf of a mottled and severely stunted cauliflower plant, horticultural variety February, selected near the center of a 10-acre planting at Alvarado. Inoculations were made to healthy February cauliflower seedlings, usually with two to three leaves, and 100-percent infection was obtained. This virus culture was used in all inoculation studies.

Inoculations were made in a greenhouse in which the temperature ranged from 13° to 19° C. The greenhouse was fumigated weekly for the control of aphids and thrips with vapors of nicotine sulphate. For the virus suspensions leaves and petioles from young cauliflower plants showing the first symptoms of the disease were ground in a sterile mortar lined with cheesecloth, and the infective juice was expressed from the macerated pulp. The carborundum method, as devised by Rawlins and Tompkins (*17*), was used.

Aging tests were conducted by pipetting 2 cc of the undiluted inoculum into small, stoppered test tubes which were stored at a constant temperature of 22° C. For determining the inactivation

temperature, 2 cc of undiluted virus extract was pipetted into small, thin-walled, stoppered test tubes, which were then immersed in an electrically heated, agitated water bath for 10 minutes at the desired temperature and cooled rapidly in running tap water. Inoculations were made immediately thereafter. Sterile, distilled water was used as the diluent in preparing the different virus concentrations employed in determining tolerance to dilution.

Since repeated inoculations with the cauliflower mosaic virus to *Nicotiana tabacum* L., *N. glutinosa* L., and *N. langsdorffii* Weinm. failed to produce infection, it was necessary to select a test host in the genus *Brassica*. Because of its marked susceptibility to infection, the February variety of cauliflower was chosen for this purpose. Seeds were planted, as needed, in flats of steam-sterilized soil. Seedlings in the two-leaf stage were transplanted to ordinary soil in 6-inch, porous clay pots. Inoculations were usually made when the seedlings had two or three leaves.

TRANSMISSION

Initial attempts in 1932 to transmit the virus to young cauliflower plants in the greenhouse by means of Holmes' (*11*) rubbing method of inoculation were unsuccessful. Later, infection was readily obtained, as shown in figure 2, by using powdered carborundum (*17*). The incubation period ranged from 10 to 20 days.

Expressed juice from roots of artificially infected February cauliflower seedlings was tested for the presence of the virus by inoculating seedlings of the same variety. Of 42 plants inoculated, 33 became infected, showing that the virus occurs in root tissues and is highly infectious. The incubation period averaged 20 days.

The problem of insect vectors of the cauliflower virus was investigated by Henry H. P. Severin of the Division of Entomology and Parasitology. He found that the following species of aphids which breed on crucifers under natural conditions are vectors of the cauliflower virus: Cabbage aphid (*Brevicoryne brassicae* (L.)), false cabbage or turnip aphid (*Rhopalosiphum pseudobrassicae* (Davis)), and green peach aphid (*Myzus persicae* (Sulzer)), and that 10 additional species of aphids which have not yet been found to breed on cauliflower under natural conditions are capable of transmitting the virus.

In April 1934, flowers produced on 12 mosaic-diseased and 12 healthy cauliflower plants, variety March, were bagged and later hand-pollinated at Alvarado. The seed from both lots was sown in an open field seedbed in August. Later, approximately 2,000 seedlings from each lot were transplanted in an isolated area. Up to harvest time, no infection was observed in either plot, which strongly suggests that the virus is not transmitted through cauliflower seed. Both lots of seed were then tested in the greenhouse under controlled conditions. None of the seedlings in either lot was diseased, substantiating field results.

It is conceivable that the cauliflower virus may be transmitted in nature by agencies other than aphids, as for example, the soil. Investigation into the possible role of such additional agencies was not undertaken.

SUSCEPTIBILITY OF CAULIFLOWER VARIETIES TO ARTIFICIAL INFECTION

All commercial varieties of cauliflower grown in California, as well as some of importance elsewhere, were tested for susceptibility by artificial inoculation in the greenhouse. Seedlings were inoculated after the first pair of true leaves had formed, while a suitable number served as controls. From the results of these tests, shown in table 1, it is apparent that all varieties are highly susceptible. Typical symptoms were shown by all infected plants of each variety tested. Although the incubation periods for varieties grown in California are within the range of 12 to 20 days, a longer period was noted for some varieties which are of commercial importance elsewhere.

TABLE 1.—*Relative susceptibility of certain varieties of cauliflower to the cauliflower mosaic virus as indicated by artificial inoculations into greenhouse-grown seedlings*

Common name or horticultural variety	Plants inoculated	Plants infected		Incubation period
	Number	*Number*	*Percent*	*Days*
April [1]	20	19	95	14–16
Autumn Giant	19	19	100	17–37
Christmas	57	57	100	19–25
Clausdale	15	15	100	27–29
Danish Dry Weather	19	17	89	21–30
Danish Perfection	20	20	100	15
Danish Snowball	20	16	80	26–35
December [1]	20	20	100	14–15
Dryweather Danish Giant	20	20	100	16–20
Early Copenhagen Snowball	20	20	100	20–29
Early Erfurt Snowball	19	17	89	25–31
Early March [1]	60	60	100	15–20
Early Snowball [1]	20	20	100	18
Extra Early Dwarf Erfurt	38	38	100	19–28
Extra Early Paris	20	20	100	21–36
February [1]	160	160	100	14–20
High Altitude Danish Snowball	20	12	60	21–36
Improved Self-Protecting Snowball	54	54	100	22–33
January [1]	20	20	100	14–16
Late March [1]	20	20	100	14
Late Pearl [1]	38	34	89	17–25
Medium Pearl [1]	20	15	75	20–31
Mission Special	20	20	100	20
Model Earliest and Best	15	10	67	21–31
November [1]	20	20	100	14–15
Premium Snow	19	13	68	31–44
Primosnow	18	18	100	26–35
Prizewinner Snowball	10	10	100	24–30
Purple Sicilian Very Early	20	20	100	19–25
Select Early Snowball	20	13	65	25–35
Snowball [1]	35	32	91	20–32
Snow King	20	20	100	25–31
Special Early	20	20	100	14–15
Special Medium	20	20	100	15
St. Valentine	20	20	100	14–15
Super Snowball [1]	20	20	100	17

[1] Varieties of commercial importance in California.

HOST RANGE

NATURAL

In addition to cauliflower, other cultivated cruciferous plants were found to be naturally infected by the cauliflower virus as proved by artificial inoculation with their expressed leaf extract to the test host in the greenhouse. These included kale (*B. oleracea* L. var. *acephala* DC.), Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), red and white varieties of cabbage (*B. oleracea* L. var. *capitata* L.), asparagus or sprouting broccoli (*B. oleracea* L. var. *botrytis* L.), and annual stock

(*Matthiola incana* R. Br. var. *annua* Voss). On these hosts, clearing of the veins was the predominant symptom; stunting was not observed.

Virus infection of wild yellow mustard (*B. campestris* L.) and wild radish (*Raphanus sativus* L.) plants is of common occurrence in cul-

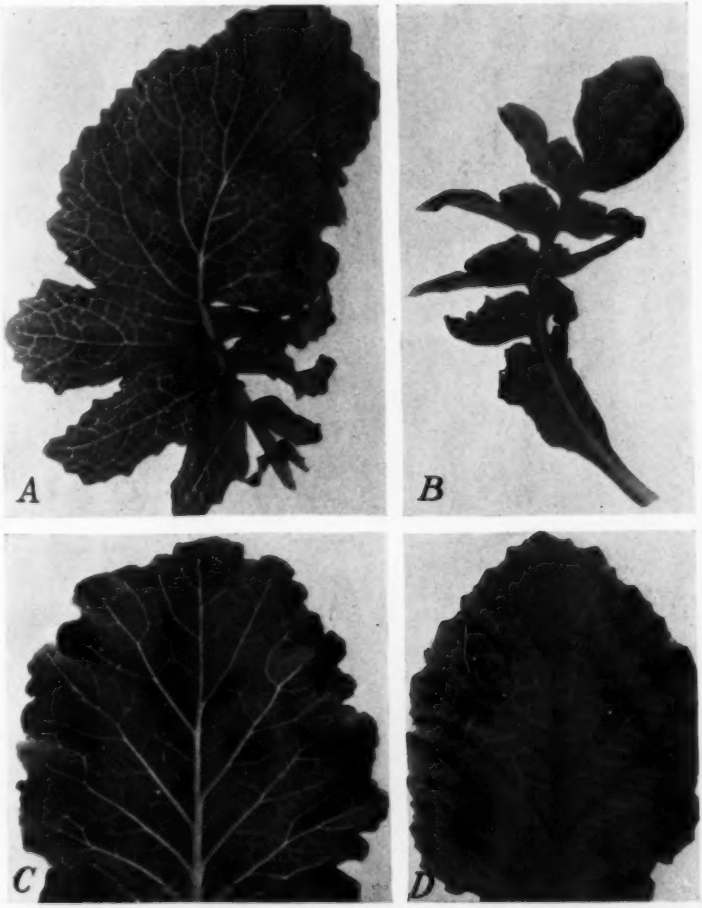

FIGURE 3.—Symptoms produced by the cauliflower mosaic virus on different crucifers after inoculation in the greenhouse at 13° to 19° C.: *A*, Wild yellow mustard; *B*, shepherds-purse; *C*, Winter Colma cabbage; *D*, Mammoth Rock Red cabbage.

tivated crucifer fields or along fence rows and ditchbanks. Generally, infected plants show a pronounced mosaic mottle on the leaves. Failure attended many trials to recover the cauliflower mosaic virus on the test host from such material. However, in January 1935, some wild mustard plants showing symptoms of vein clearing on the leaves were collected at the edge of a field of sprouting broccoli near San Jose, from which the cauliflower virus was readily recovered.

EXPERIMENTAL

Results of artificial inoculation tests in the greenhouse are given in table 2. Vein-clearing symptoms were shown by all plants experimentally infected (figs. 3 and 4). In all cases, the virus was recovered on the test host to establish proof of infection.

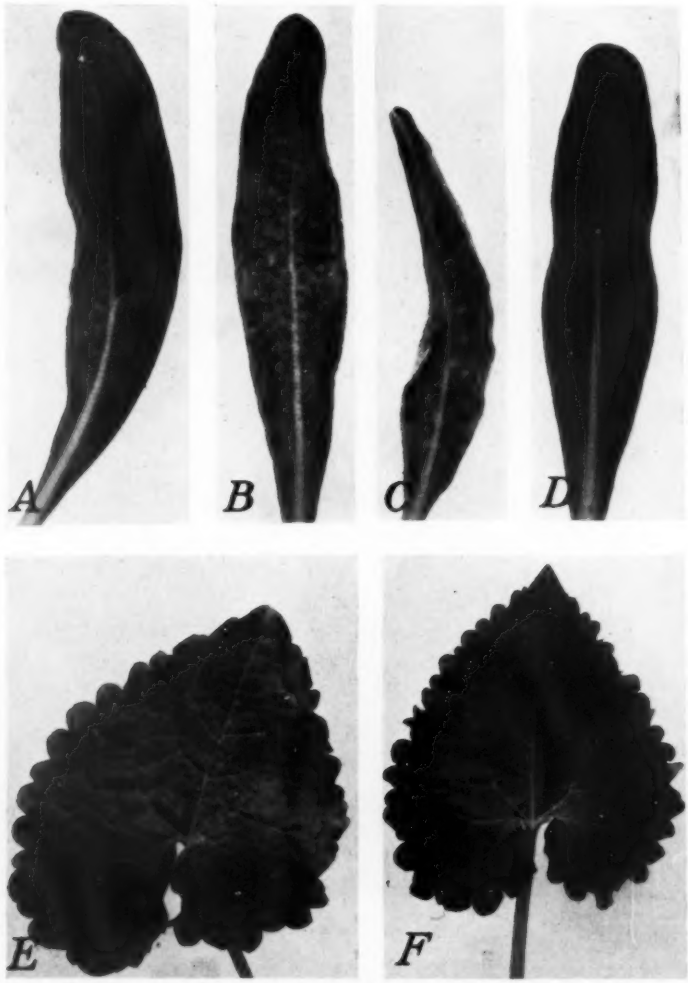

FIGURE 4.—Vein-clearing symptoms produced on leaves of ornamental crucifers by the cauliflower mosaic virus after artificial inoculation in the greenhouse at 13° to 19° C.: *A, B, C,* Infected leaves of annual stock (*Matthiola incana* var. *annua*); *D,* noninoculated control; *E,* infected leaf of honesty (*Lunaria annua*); *F,* noninoculated control.

Infected annual stock (*Matthiola incana* var. *annua*) did not show breaking of the flowers, or petal variegation, a disease recently reported by Tompkins (*22*). Breaking of the flowers of annual stock is

caused by a different crucifer virus, which in turn does not infect cauliflower or cabbage (*22*).

TABLE 2.—*Relative susceptibility of certain cultivated and wild crucifers to the cauliflower mosaic virus as indicated by artificial inoculations into greenhouse-grown seedlings*

Species and common name	Horticultural variety	Plants inoculated	Plants infected		Average incubation period
		Number	*Number*	*Percent*	*Days*
Cultivated hosts:					
Brassica oleracea L. var. *acephala* DC. (kale).	Dwarf Green Curled	20	20	100	15
	Jersey	20	20	100	18
	Siberian or Sprouts	20	20	100	20
	Tall Green Curled Scotch	20	9	45	25
	White Marrow	18	17	94	17
B. oleracea L. var. *gemmifera* DC. (Brussels sprouts).	Long Island Improved	20	18	90	26
	Selected	20	17	85	35
B. oleracea L. var. *capitata* L. (cabbage).	All Seasons	20	11	55	31
	Charleston or Large Wakefield	20	20	100	17
	Copenhagen Market	20	20	100	15
	Danish Ballhead	20	12	60	23
	Early Dwarf Flat Dutch	20	15	75	30
	Early Winningstadt	20	20	100	16
	Glory of Enkhuizen	16	16	100	25
	Hollander	16	15	94	21
	Mammoth Rock Red	20	20	100	21
	Perfection Drumhead Savoy	20	20	100	17
	Round Red Dutch	20	20	100	21
	San Francisco Market	20	20	100	17
	Wisconsin All Seasons	20	12	60	27
	Wisconsin No. 8	20	17	85	27
	Winter Colma	20	20	100	17
B. oleracea L. var. *botrytis* L. (asparagus or sprouting broccoli).	Italian Green Sprouting	20	19	95	18
B. oleracea L. var. *caulorapa* DC. (kohlrabi).	Early Purple Vienna	10	4	40	24
	Early White Vienna	20	20	100	24
B. napus L. (rape)		15	15	100	18
B. campestris L. var. *napobrassica* DC. (rutabaga).	American Purple Top	20	20	100	15
	Harleys Bronze Top	20	20	100	15
	Large White	20	20	100	19
	Monarch or Tankard	20	20	100	13
B. rapa L. (turnip)	Early White Flat Dutch	20	20	100	22
	Purple Top White Globe	20	20	100	18
	Purple Top Yellow Aberdeen	7	7	100	34
	Seven Top	20	14	70	22
	Shogoin or Japanese	18	18	100	19
B. juncea Coss (leaf mustard)	Chinese	20	20	100	14
	Florida Broad Leaved	7	7	100	32
B. pe-tsai Bailey (pe-tsai)	Fordhook Fancy	20	17	85	17
	Spinach or Tendergreen	8	8	100	27
	Chinese pe-tsai	20	18	90	17
	Pe-tsai (Hootooren)	20	20	100	19
	Pe-tsai (Nagasaki Hakusai)	20	20	100	20
	Pe-tsai (Tsuzita Hakusai)	20	20	100	14
B. nigra Koch (black mustard)		10	10	100	29
B. alba Rabenh. (white mustard)		10	7	70	27
Matthiola incana R. Br. var. *annua* Voss (annual stock).	Empress Augusta Victoria	6	6	100	65
	Fiery Blood Red	10	8	80	70
Lunaria annua L. (honesty)		20	20	100	51
Lepidium sativum L. (garden cress).	Curled or Pepper Grass	10	6	60	32
Iberis amara L. (rocket candytuft).		10	3	30	33
Raphanus sativus L. (radish)	Early White Giant Summer	8	4	50	72
	French Breakfast	7	3	43	72
	Long Black Spanish	7	2	29	34
	Long White Japanese	8	4	50	72
	Scarlet Turnip	20	20	100	30
	White Icicle	20	19	95	27
Wild hosts:					
B. adpressa Boiss.		12	11	92	32
B. arvensis (L.) Ktze. (charlock)		25	10	40	61
B. campestris L. (wild yellow mustard).		4	4	100	18
Capsella bursa-pastoris Medic. (shepherds-purse).		10	10	100	29
Raphanus raphanistrum L. (jointed or white charlock).		8	4	50	72

Up to the present time and as far as these studies were carried, no plants other than crucifers have been experimentally infected. Studies on this phase of the problem, to include the testing of a large number of species belonging to other families, are now in progress.

In addition to wild yellow mustard (*B. campestris*) and wild radish (*R. sativa*), infection of other wild crucifers was obtained in the greenhouse, including *B. adpressa* Boiss, *B. arvensis* (L.) Kuntze (charlock), *Capsella bursa-pastoris* (L.) Medic. (shepherds-purse) (fig. 3, *B*), and *R. raphanistrum* L. (jointed or white charlock). It is suggested they may be important as sources of inoculum and as means for overwintering of the virus under natural conditions. This problem is receiving further attention.

The experimental host range, as determined by inoculations, includes 51 vegetable varieties, 3 ornamentals, and 5 wild crucifers.

NEGATIVE REACTIONS

Unsuccessful attempts were made to transmit the cauliflower virus by artificial inoculation to spinach (*Spinacia oleracea* L.) var. Bloomsdale, leaf mustard (*B. juncea* Coss) from Gifu, Japan, rockcress (*Arabis albida* Stev.), wallflower (*Cheiranthus cheiri* L.), blistercress (*Erysimum perofskianum* Fisch. and Mey.), dames violet (*Hesperis matronalis* L.), Virginian stock (*Malcomia maritima* R. Br.), water cress (*Roripa nasturtium* Rusby), golden tuft (*Alyssum saxatile* L.), sweet alyssum (*Alyssum maritimum* Lam.), *Athysanus pusillus* Greene, *Stanleya pinnata* (Pursh.) Britt., *Thysanocarpus radians* Benth., broadbean (*Vicia faba* L.), garden nasturtium (*Tropaeolum majus* L.), celery (*Apium graveolens* L.), tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey), currant tomato (*L. pimpinellifolium* Dunal), bell or sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.), *Nicotiana tabacum* (var. Turkish and White Burley), *N. glutinosa*, *N. langsdorffii*, head lettuce (*Lactuca sativa* L. var. *capitata* Hort.), and cos lettuce (*L. sativa* var. *romana* Hort.).

PROPERTIES OF THE VIRUS

As shown in table 3, the cauliflower virus retained its infectivity in one trial after storage at a constant temperature of 22° C. for 14 days (336 hours), but was inactivated at the end of 15 days (360 hours). Under conditions of the tests, its longevity in vitro lies between 14 and 15 days. The virus has an inactivation temperature at or near 75° for 10 minutes. A dilution tolerance of approximately 1 to 2,000 was determined for the virus.

DESCRIPTION OF THE CAULIFLOWER MOSAIC VIRUS

Transmitted in nature by *Brevicoryne brassicae* L., *Rhopalosiphum pseudobrassicae* Davis, and *Myzus persicae* Sulzer. Transmissible by artificial inoculation with expressed juice and powdered carborundum. Incubation period 10 to 20 days. Resistance to aging in vitro between 14 and 15 days. Inactivation temperature at or near 75° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 2,000. Cauliflower (*Brassica oleracea* L. var. *botrytis* L.) and certain other vegetable and ornamental crucifers susceptible. On cauliflower, symptoms consist of stunting of plant; vein clearing followed by vein banding, mottling, necrotic spotting, curvature of midrib, and distortion of leaves; dwarfing of terminal head or curd. *Nicotiana tabacum* L., *N. glutinosa* L., and *N. langsdorffii* Weinm. not infected.

TABLE 3.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the cauliflower mosaic virus*

LONGEVITY IN VITRO, 22° C.

Aged (hours)	Trials	Plants inoculated	Plants infected	Aged (hours)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	25	168	5	25	25
4	5	25	25	192	5	25	25
8	5	25	25	216	5	25	25
12	5	25	25	240	5	25	20
24	5	25	25	264	5	25	16
48	5	25	25	288	5	25	15
60	5	25	25	312	5	25	6
72	5	25	25	336	5	25	3
96	5	25	25	360	5	25	0
120	5	25	25	384	5	25	0
144	5	25	25				

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (° C.)	Trials	Plants inoculated	Plants infected	Temperature (° C.)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
Not heated, used immediately	5	25	25	70	5	25	25
50	5	25	25	75	5	25	0
55	5	25	25	Not heated, used 3 hours after extraction	5	25	25
60	5	25	25				
65	5	25	25				

TOLERANCE TO DILUTION

Dilution	Trials	Plants inoculated	Plants infected	Dilution	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	25	1:500	5	25	25
1:10	5	25	25	1:1,000	5	25	14
1:25	5	25	25	1:2,000	5	25	8
1:50	5	25	25	1:3,000	5	25	0
1:100	5	25	25	1:4,000	5	25	0
1:200	5	25	25				

COMPARISON OF THE CAULIFLOWER MOSAIC VIRUS WITH A TURNIP MOSAIC VIRUS FROM NEW YORK

An earlier suggestion by the writer that the rutabaga virus is different from the cauliflower virus was based entirely upon Clayton's (*3*) description of symptoms and photographs. Comparative inoculation tests could not be made since Clayton's rutabaga virus was not available. However, a mosaic disease of white turnip which causes serious losses in stecklings intended for seed propagation was prevalent in 1934 on Long Island, N. Y., specimens of which were obtained.[7] The virus was readily established on young Purple Top White Globe turnip plants in the greenhouse. Conceivably the turnip virus may be closely related to or even identical with the rutabaga virus (*3*), but experimental evidence is lacking. In a later communication,

[7] Diseased turnips were sent through the courtesy of J. M. Lupton & Son, Inc., Mattituck, Long Island, N. Y.

Clayton [8] stated he had never observed a mosaic disease of turnips on Long Island.

It is interesting to note that after young Winter Colma cabbage and February cauliflower plants had been inoculated with the turnip virus, leaf symptoms developed (fig. 5, *A*, *B*) which were not unlike those induced on Brussels sprouts and cauliflower by the rutabaga virus as depicted by Clayton (*3*) in his figure 1, *B*, *C*, on page 267. The leaf symptoms on cauliflower consisted of slight chlorosis, with small, light-green to yellow circular areas, varying in diameter from 1/16 to 3/16 of an inch, distributed between or adjacent to the veins of the leaf. On cabbage, the lesions were of the same general type, but later the edges became necrotic.

Successful inoculations with the turnip virus were made only to six additional varieties of cauliflower: April, Danish Perfection,

FIGURE 5.—Leaf symptoms produced by a turnip mosaic virus from New York after artificial inoculation in the greenhouse at 13° to 19° C.: *A*, Light-green to yellow circular lesions, becoming necrotic around the edges with age, on Winter Colma cabbage; *B*, pale-green to yellow circular lesions on February cauliflower.

Dry-weather Danish Giant, Early March, Early Snowball, and Super Snowball. The incubation period ranged from 15 to 20 days.

Infection was also obtained on Brussels sprouts, rutabaga, pe-tsai, annual stock (in addition to mottled leaves, the flowers showed breaking), *Nicotiana tabacum* (var. Turkish), and *N. glutinosa*. Asparagus or sprouting broccoli, kohlrabi, radish, and *N. langsdorffii* were not infected.

These limited tests indicate that the turnip virus is different from the cauliflower virus.

SUMMARY

A mosaic disease of cauliflower, caused by a hitherto undescribed virus, is reported in this paper.

The disease occurs chiefly in the cool, coastal valleys of California, frequently causing considerable loss to the crop.

[8] Letter dated Mar. 25, 1936, from E. E. Clayton, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

Cauliflower mosaic is characterized by stunting of the plant; vein clearing followed by vein banding, mottling, necrotic spotting, curvature of midrib, and distortion of the leaves; and dwarfing of the terminal head or curd.

The insect vectors are *Brevicoryne brassicae*, *Rhopalosiphum pseudobrassicae*, and *Myzus persicae*. The cauliflower virus is also transmissible by artificial juice inoculations when carborundum is used as an abrasive. The virus withstands aging in vitro for 14 to 15 days at 22° C. Its inactivation temperature is approximately 75°, while the tolerance to dilution is about 1 to 2,000.

The host range includes 51 vegetable varieties, 3 ornamentals, and 5 weeds, all belonging to the family Cruciferae.

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RELATION OF CERTAIN DIETARY ESSENTIALS TO FERTILITY IN SHEEP[1]

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INTRODUCTION

Economic studies of the sheep industry show that the percentage lamb crop is the most important single factor in the cost of production. This percentage is usually figured on the number of live lambs born as compared to the number of ewes bred. The number of lambs produced per hundred ewes depends, of course, to some extent on the number of barren ewes and the number producing twins and triplets. Heape[2] gathered statistics on this problem from flockmasters through the Royal Agricultural Society of England. Fertility as recorded by 306 flockmasters ranged from 203.8 to 59.09 percent, and the proportion from 107,603 ewes was 120.4 percent. An analysis of this figure showed that an average of 30 ewes produced twins and 60 ewes produced singles, 90 ewes thus producing 120 lambs; 3 ewes died; and 7 were barren or aborted. The number of lambs produced by 100 ewes was, therefore, 120. Under range conditions in California the usual lamb crop is 80 to 90 percent.

One hundred and twenty-five high-grade yearling Rambouillet ewes were purchased by the University of California in 1927 and 80 Romney-Rambouillet cross-bred ewes were purchased in 1928 for cross-breeding experiments[3] in the production of spring lambs. The Rambouillet ewes came from a long-established flock in which purebred Rambouillet rams had been used for 25 years. The flock, which numbered 6,000 to 8,000 ewes, had ranged throughout the year on the west side of the San Joaquin Valley. It had a lambing record of 80 to 100 percent. The Romney-Rambouillet ewes came from a flock of about 2,000 head which had ranged throughout the year in the Sacramento Valley. The average lamb crop in this flock was about 95 percent. When these ewes came to the experiment station, where feed conditions were better during most of the year, the average production of the Rambouillet ewes rose to 135.5 percent and that of the Romney–Rambouillets to 127.6 percent for the 6 and 4 years respectively that the ewes were in the breeding experiment. For the 3 years in which the ewes were in their prime several lots produced a 160- to 165-percent lamb crop.

These observations suggested the advisability of ascertaining whether any particular dietary essential was necessary for high fertility or whether the problem was merely one of total energy intake.

[1] Received for publication Oct. 29, 1936; issued August 1937. Part of an investigation of the relation of nutrition to reproduction which has been carried on in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture, since July 1, 1929.

[2] HEAPE, W. ABORTION, BARRENNESS, AND FERTILITY IN SHEEP: AN ABSTRACT OF RECORDS OBTAINED FOR THE YEAR 1896–97. Jour. Roy. Agr. Soc. England (3) 10: [217]–248. 1899.

[3] MILLER, R. F. CROSSBREEDING INVESTIGATION IN THE PRODUCTION OF CALIFORNIA SPRING LAMBS. Calif. Agr. Expt. Sta. Bull. 598, 32 pp., illus. 1935.

EXPERIMENTAL DATA

RELATION OF VITAMIN A TO FERTILITY

Observations on the feeding of breeding ewes had led the writers to believe that vitamin A deficiency might be a factor limiting fertility under practical conditions. An experiment was, therefore, undertaken to determine the influence of vitamin A on fertility. On May 9, 1932, the lambs from 72 of the Romney–Rambouillet ewes used in the cross-breeding experiment mentioned above were weaned and the ewes placed in dry lot on oat hay and a small amount of grain mixture. On May 14 the ewes were segregated into 3 lots of 24 each and fed as follows:

Lot 1. Low vitamin A diet: Oat hay and grain mixture in dry lot.

Lot 2. Low vitamin A diet plus cod-liver oil during breeding: Oat hay and grain mixture in dry lot.

The grain mixture consisted of 40 pounds of dried molasses beet pulp, 20 pounds of cottonseed meal, 20 pounds of rolled barley, and 10 pounds of wheat bran.

Lot 3. Control group maintained on pasture according to usual farm practice. The term "usual farm practice" as used in this paper means that the ewes are maintained in summer on dry native pasture alternated with irrigated Sudan or alfalfa pasture and in winter on native green pasture supplemented with alfalfa hay, silage, or barley.

The experimental animals in these and all subsequent tests had access to brick salt at all times. On June 10, 10 percent of dry skim-milk powder was added to the grain mixture. In the period from June 18 to 22 the oat hay was gradually withdrawn, cottonseed hulls substituted, and the grain mixture increased so that 2 pounds of hulls and one-half pound of grain per ewe were supplied each day.

On August 1, lot 3 was taken from native dry pasture and placed on green Sudan grass during the day. At the same time the administration of cod-liver oil was started in lot 2, each animal receiving daily 10 cc from a pipette directly into the mouth.

On August 6 one ewe was removed from each lot because of poor physical condition. The following day two 2-year-old Hampshire rams were turned in and rotated among the three lots of ewes.

One ewe in lot 2 was accidentally killed on August 20.

On October 7 the breeding period closed, the rams were removed, and the administration of cod-liver oil to lot 2 was stopped. Lots 1 and 2 were both in good condition, but the ewes in lot 3 were somewhat heavier.

During gestation all three lots were handled according to the usual practice on the farm.

During lambing in January the ewes were fed a grain mixture and alfalfa hay. Corn silage was added to this ration for the group that had lambs a week or more old.

On February 1 lambing was about over, the pasture was still short, and the ewes stayed in the corral most of the time. By March 1 native pasture was good and grain feeding was discontinued. The lambs were weaned on April 23. The lambing records of these lots are given in table 1 and the weights of the ewes in table 2.

It will be observed that the ewes lost weight after the lambs were weaned in May. They became quite accustomed to the restricted rations and during the breeding season from August 6 to October 7 the average gain of the three lots was 10.3, 17.1, and 23.4 pounds per head, respectively.

TABLE 1.—*Effect of variations in the vitamin A content of the ration during breeding on the lambing record of ewes, 1933*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb [2] crop
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	23	19	3	0	1	25	108.7
2	22	14	5	1	2	27	122.7
3	23	9	13	0	1	35	152.2

[1] 1 single lamb died shortly after birth in lot 1. In lot 2 the ewe with triplets died before parturition, 1 pair of twins and 1 single lamb were born dead, and 1 single died shortly after birth. In lot 3, 5 twin lambs, and 2 singles died soon after birth.

[2] Based on the number of lambs alive or dead in relation to the number of ewes bred.

TABLE 2.—*Effect of variations in the vitamin A content of the ration during breeding on the weight of ewes, 1932*

Date (1932)	Lot no.	Ewes	Average weight	Gain or loss per week per ewe	Date (1932)	Lot no.	Ewes	Average weight	Gain or loss per week per ewe
		Number	*Pounds*	*Pounds*			*Number*	*Pounds*	*Pounds*
	1	24	147.9	----------		1	24	135.4	−0.9
May 14	2	24	147.9	----------	July 30	2	24	136.7	+1.3
	3	24	147.9	----------		3	24	146.0	−4.6
	1	24	146.6	−1.3		1	23	139.5	+4.1
May 21	2	24	146.6	−1.3	Aug. 6	2	23	136.1	−.6
	3	24	145.4	−2.5		3	23	137.0	−9.0
	1	24	144.0	−2.6		1	23	139.1	−.4
May 28	2	24	144.0	−2.6	Aug. 13	2	23	137.8	+1.7
	3	24	143.7	−1.7		3	23	139.1	+2.1
	1	24	142.9	−1.1		1	23	144.1	+5.0
June 4	2	24	142.5	−1.5	Aug. 20	2	22	139.8	+2.0
	3	24	143.3	−.4		3	23	140.4	+1.3
	1	24	144.7	+1.8		1	23	143.0	−1.1
June 11	2	24	145.0	+2.5	Aug. 27	2	22	143.6	+3.8
	3	24	145.0	+1.7		3	23	147.2	+6.8
	1	24	137.1	−7.6		1	23	144.3	+1.3
June 18	2	24	137.1	−7.9	Sept. 3	2	22	145.9	+2.3
	3	24	139.2	−5.8		3	23	147.8	+.6
	1	24	140.0	+2.9		1	23	146.3	+2.0
June 25	2	24	140.0	+2.9	Sept. 10	2	22	147.7	+1.8
	3	24	142.1	+2.9		3	23	149.6	+1.8
	1	24	139.8	−.2		1	23	141.7	−4.6
July 2	2	24	140.2	+.2	Sept. 17	2	22	143.8	−3.9
	3	24	140.0	+2.1		3	23	150.9	+1.3
	1	24	139.6	−.2		1	23	144.5	+2.8
July 9	2	24	142.5	+2.3	Sept. 24	2	22	148.4	+4.6
	3	24	----------	----------		3	23	151.3	+.4
	1	24	136.7	−2.9		1	23	149.3	+4.8
July 16	2	24	137.5	−5.0	Oct. 1	2	22	151.3	+2.9
	3	24	----------	----------		3	23	157.6	+6.3
	1	24	136.3	−.4		1	23	149.8	+.5
July 23	2	24	135.4	−2.1	Oct. 7	2	22	153.2	+1.9
	3	24	150.6	----------		3	23	160.4	+2.8

The lamb crop of lot 3, kept under regular farm conditions, was in line with that during the period when they were in the previously mentioned cross-breeding experiments. The restricted diet of lots 1 and 2 had an adverse effect on the lamb crop. Very low vitamin A intake from May 14 to October 7 in lot 1 did not significantly lower the number of lambs as compared to that in lot 2. The addition of the 10 cc of cod-liver oil daily to lot 2 during the breeding period had no significant effect on reproduction, but it may have been a factor in the increased weight gain over the animals in lot 1. The cod-liver oil used in this work when later tested did not prove to be as potent as claimed on the label, but the 10-cc daily doses contained enough of this factor to furnish at least twice the minimum requirement.

RELATION OF VITAMIN A AND PROTEIN TO FERTILITY

In 1933–34, the second year of the study, 7-year-old grade Rambouillet ewes taken from the cross-breeding experiment mentioned above, were put on a diet low in protein and kept on the diet until the close of the breeding season.

The lambs were weaned on April 30, and 80 of the ewes were placed in dry lot on oat-hay and grain mixture.

On June 8 they were divided into four lots, each containing 20 head, and fed as follows:

Lot 1. Low vitamin A diet.
Lot 2. Low vitamin A diet plus cod-liver oil during breeding.
Lot 3. Low vitamin A diet plus Sudan grass pasture during breeding.
Lot 4. Low vitamin A and low protein diet plus cod-liver oil during breeding.

For the first three lots the roughage was a proprietary feed, 2 pounds per head daily, and one-half pound daily per ewe of a grain mixture consisting of 60 pounds of dried molasses beet pulp, 20 pounds of rolled barley, and 20 pounds of wheat bran. The proprietary feed consisted of 60 pounds of cottonseed hulls, 25 pounds of cottonseed meal, and 15 pounds of cane molasses.

Lot 4 received oat hay, 2 pounds daily per ewe, with one-half pound daily of a grain mixture containing 75 pounds of dried molasses beet pulp and 25 pounds of rolled barley.

From July 7 to 16, lot 4 was gradually changed from the oat to hay cottonseed hulls. The total protein intake was thereby reduced from 8 to 5.5 percent. The protein in the hulls is only 20 percent digestible.

One ewe in each of lots 1 and 3 died on July 1. On July 27 a second ewe in lot 1 became sick and was destroyed. Her liver contained 2,500 units of vitamin A.[4]

On August 1 all of the lots began to receive cottonseed hulls, as the supply of the proprietary meal was exhausted. This made it necessary on that date to change the grain mixture for lots 1, 2, and 3 to one made of 50 pounds of dried molasses beet pulp, 30 pounds of cottonseed meal, 10 pounds of rolled barley, and 10 pounds of wheat bran.

On August 19 one ewe from lot 2 was killed for the determination of liver storage of vitamin A, and it was found to contain 4,000 units. This showed that the high vitamin A store in these aged ewes was not being depleted very rapidly even though their diets had been deficient in this substance for 3½ months.

On September 2 the plan of the experiment was therefore changed somewhat. Four ewes from each of lots 1, 2, and 3 and six ewes from lot 4 were eliminated from the experiment. This left 18 head to be kept on low vitamin A rations for determining how long their reserves would carry them. These ewes were killed at intervals of about 2 months and their livers examined. Withdrawal of vitamin A from the liver was found to have been much more rapid during the first year than during the second. The last of the ewes, just maintaining themselves without the drain of reproduction and lactation, lived for over 2 years on the vitamin A deficient diet, and night blindness did not appear until after the lapse of 22 to 23 months.

The four original lots now contained 14 ewes each, except lot 2, which contained 15. Ten cubic centimeters of cod-liver oil per ewe was

[4] This is an antimony trichloride blue unit of vitamin A estimated by the technique outlined by Guilbert and Hart [5] and approximates 0.1 microgram of vitamin A and respresents 0.25 rat unit U. S. P. 1934 revised.

[5] GUILBERT, H. R., and HART, G. H. STORAGE OF VITAMIN A IN CATTLE. Jour. Nutrition 8: 25–44. 1934.

given daily by pipette, beginning September 2, to the animals in lots 2 and 4. This cod-liver oil and that subsequently used contained 2,100 United States Pharmacopoeia 1934 revised units per gram. Tests with the Hilger vitameter A showed it to contain 0.083 percent of vitamin A.

Lot 3 was turned out on barley stubble and alfalfa pasture with some Sudan-grass hay on September 2 for 3 or 4 days, and then on Sudan-grass pasture. On September 10 two Rambouillet rams were turned in and rotated between the groups. By October 12 all the ewes seemed to have been bred. The cottonseed hulls and grain mixture were stopped in all lots and cod-liver oil feeding was stopped in lots 2 and 4.

All the ewes were now fed alfalfa hay and turned out on green feed for about 1 hour a day, the time being gradually increased for a week, after which they were left on the green feed.

On October 26 all lots were turned out with the main farm flock. Alfalfa-hay feeding, one-half pound per head daily, was started on December 20 and on January 2 a grain-mixture feeding was begun.

Lambing began on February 9. The lambing data for these groups are given in table 3 and the weights of the ewes in table 4.

TABLE 3.—*Effect of variations in the vitamin A and protein content of the ration during breeding on the lambing record of ewes, 1934*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop [2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	14	5	6	2	1	23	164.3
2	15	6	8	1	0	25	166.6
3	14	3	9	0	2	21	150.0
4	14	6	5	1	2	19	135.7

[1] In lot 1, 2 lambs of 1 set of triplets were born dead and 1 single died shortly after birth. In lot 2, 2 of the triplets and 2 singles were born dead; 1 ewe with prolapsed vagina died and twin lambs were found in her uterus. In lot 3, 1 single lamb was born dead and 1 twin died shortly after birth. In lot 4, 1 single lamb was born dead.

[2] See footnote 2, table 1.

TABLE 4.—*Effect of variations in the vitamin A and protein content of the ration during breeding on the weight of ewes, 1933*

Date (1933)	Lot no.	Ewes	Average weight	Gain or loss per ewe	Date (1933)	Lot no.	Ewes	Average weight	Gain or loss per ewe
		Number	*Pounds*	*Pounds*			*Number*	*Pounds*	*Pounds*
June 8	1	20	141.6		Sept. 30	1	14	148.4	−2.7
	2	20	145.9			2	15	146.1	−2.4
	3	20	147.1			3	14	157.6	+6.2
	4	20	152.0			4	14	148.9	+3.4
June 29	1	18	156.7	+15.1	Oct. 12	1	14	153.7	+5.3
	2	20	154.2	+8.3		2	15	148.1	+2.0
	3	19	154.5	+7.4		3	14	159.0	+1.4
	4	20	156.0	+4.0		4	14	148.6	− .3
Sept. 1	1	18	151.1	−5.6					
	2	19	148.5	−5.7					
	3	18	151.4	−3.1					
	4	20	145.5	−10.5					

The results of this experiment coupled with that of the previous year led to the belief that the animals were not being at all seriously depleted of vitamin A by the methods used. Slaughtered ewes

showed a high vitamin A concentration in the liver. The 5-months period on a low vitamin A diet did not deplete the storage in the old ewes as anticipated. That the vitamin A reserves were not soon reduced was further evidenced by the length of life of the group that was continued on the low intake levels for nearly 2 years before night blindness, the first symptom of vitamin A deficiency, appeared.

The breeding period in 1933 extended from September 10 to October 12, and the average weight change per ewe from September 1 to October 12 in each lot was +2.6, −0.4, +7.6, and +3.1 pounds, respectively, which was not enough according to the general empirical requirements to be considered true flushing. Nevertheless, lot 1 on the vitamin A-low diet with a small gain per ewe produced a 164.3-percent lamb crop. This was not significantly different from that of the ewes of lot 2 which had the same ration as lot 1 plus the cod-liver oil and only maintained their weight during the breeding period. It is therefore apparent that actual gain in weight is not necessary for a high percentage lamb crop provided the ewes are in sufficiently good condition and have enough body stores of the essential food substances.

Lot 3, which was kept on green Sudan grass pasture during breeding and which made the largest gain, did not give birth to as many lambs as lots 1 and 2, but still produced a 150-percent lamb crop, which is very high.

Lot 4 produced a highly satisfactory lamb crop, even though it was the lowest of the four groups and its rations were the most severely restricted.

From the foregoing results it was concluded that the problem would have to be studied with young and growing ewes that had not had the opportunity to store large reserves of the needed dietary essentials in their bodies. Accordingly, it was decided to discard all adult ewes and begin the next year with ewe lambs only.

RELATION OF VITAMIN A TO FERTILITY IN EWE LAMBS

In 1934–35, the third year of the study, vitamin A alone was restricted as in the first year, with the control group maintained under the usual farm conditions. Contrary to the procedure followed in the first year, the low vitamin A diet was maintained throughout the gestation period.

Sixty-one lambs averaging 62 pounds in weight were purchased on June 21, 1934. They were turned on barley stubble until July 24, when they were divided into 2 lots of 30 head each and placed on the following rations:

Lot 1. Low vitamin A diet: Cottonseed hulls and the grain mixture used the previous year after the proprietary meal had been stopped.
Lot 2. Placed on Ladino clover pasture.

The one extra animal was a wether lamb, which was killed July 25. Its liver contained 750 units of vitamin A per gram.

One lamb in lot 2 died on August 29.

On October 22 one lamb from each group was killed and the livers examined for vitamin A. The sample of liver from the lamb of lot 1 contained 1,000 units and that of the lamb from lot 2 contained 1,250 units of vitamin A per gram of tissue, thus showing considerable storage.

For the breeding which started on October 26, a 2-year-old Southdown ram was used. On that date, to facilitate breeding, lot 2 was placed in the corral. This lot was fed the grain mixture and alfalfa hay. On November 1 the animals in lot 1 were thin and not vigorous as compared to the thrifty animals in lot 2.

On December 1 the ram was removed after having been with the ewes for 36 days. Lot 1 was continued on the low vitamin A diet during the gestation period and lot 2 was handled according to the usual practice with the main farm flock. On January 1 the animals in lot 1 were thin as compared to those in lot 2 and on February 1 they still did not appear thrifty.

On February 15 one ewe from each lot was killed for the determination of vitamin A. The animal from lot 1 had 250 units of vitamin A per gram of liver tissue and the animal from lot 2 had 3,300 units.

Of the animals in lot 1, two showed definite and one showed partial night blindness on March 21. Lambing began on that date.

On March 29 one pregnant ewe in lot 1 had a prolapsed uterus. She was killed and her liver was found to contain 25 units of vitamin A per gram of tissue.

On April 8 nine head of lot 1 were night-blind. These were placed in individual pens and fed individually definite amounts of carotene, cod-liver oil, or dehydrated alfalfa meal for a study of the minimum requirements of vitamin A.

On April 20 the remaining 18 ewes in lot 1 were partially or completely night-blind. Nine of these were kept corralled and fed as a group definite quantities of dehydrated alfalfa meal of known carotene content. On April 29 the remaining nine ewes in lot 1 were allowed green feed in addition to a small amount of grain, and on May 7 they were found to have recovered completely normal vision.

The last lamb from the lot 1 ewes was born on April 15 and the last from the lot 2 ewes on April 24.

The lambing data are given in table 5 and the weights of the ewes in table 6.

TABLE 5.—*Effect of vitamin A depletion and of variations in the vitamin A content of the ration during breeding and gestation on the lambing record of ewe lambs, 1935*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop [2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	28	8	0	0	20	8	28.6
2	27	13	0	0	14	13	48.1

[1] In lot 1, 7 lambs died shortly after birth. In lot 2, 1 lamb was born dead and 1 died shortly after birth.
[2] See footnote 2, table 1.

TABLE 6.—*Effect of vitamin A depletion and of variations in the vitamin A content of the ration during breeding and gestation on the weight of ewes, 1934–35*

Date	Lot no.	Ewes	Average weight	Gain or loss per ewe	Date	Lot no.	Ewes	Average weight	Gain or loss per ewe
(1934)		*Number*	*Pounds*	*Pounds*	(1935)		*Number*	*Pounds*	*Pounds*
June 21	1	30	62.0	----------	Jan. 2	1	29	75.7	+3.0
	2	30	62.0	----------		2	28	85.6	−3.7
Oct. 1	1	30	67.4	+5.4	Feb. 2	1	29	84.8	+9.1
	2	29	81.5	+19.5		2	28	91.3	+5.7
Nov. 1	1	29	69.0	+1.6	Mar. 2	1	28	87.7	+2.9
	2	28	86.8	+5.3		2	27	96.1	+4.8
Dec. 1	1	29	72.7	+3.7	Apr. 1	1	27	80.2	−7.5
	2	28	89.3	+2.5		2	27	101.1	+5.0

From table 6 it will be observed that from October 1, 26 days before breeding began, and during the 36 days of the breeding period, October 26 to December 1, the ewes of lots 1 and 2 made an average gain of 5.3 and 7.8 pounds, respectively.

The most interesting result of this year's experiment was occasioned by continuing the ewes of lot 1 on a low vitamin A ration during the gestation period. This demonstrated that even young growing ewe lambs that had had access to one season of green feed as nursing lambs stored sufficient vitamin A to enable them to remain on a low vitamin A diet from June 21, 1934, to March 21, 1935, a period of 9 months, before night blindness occurred. When breeding started on October 26 these ewes were not much lower in vitamin A than the lot 2 animals, as determined by liver analysis of one animal from each group. The animals of lot 1 did not do well on the vitamin A-deficient ration and did not have as high a percentage of pregnancies as those of lot 2. Lot 2 was subjected to more favorable conditions than lot 1 and the animals were more thrifty. This probably accounts for the larger lamb crop. Vitamin A cannot be considered the cause of this difference as the data for the next breeding season clearly shows (table 7). No ewe in either lot had twins or triplets. The very significant fact is that in lot 1 the lambs were born weak and all but one died within 5 days while in lot 2, 1 lamb was born dead, 1 died shortly after birth, and the remaining 11 were normal. Since ewe lambs have comparatively few oestrous cycles during the breeding season,[6] more or less difficulty in reproduction and a low percentage lamb crop are to be expected from breeding these animals.

From evidence presented it is clear that vitamin A is highly important in gestation. A high death rate of fetuses in the uterus and a high mortality of living offspring, which are usually born weak, is to be expected if stores of vitamin A become too low in ewes toward the end of gestation.

RELATION OF VITAMIN A, PROTEIN, AND PHOSPHORUS TO FERTILITY

In 1935–36, the fourth year of the study, vitamin A restriction was continued in order to have a group of animals still more severely depleted of this factor at breeding time. In addition, one group of animals was maintained on a low protein and phosphorus ration and one on a low protein ration.

The 54 head that remained from the ewe lambs of the previous year were used. On June 8 they were divided into 3 lots each consisting of 18 head to be handled as follows:

Lot 1. Low vitamin A diet: Cottonseed hulls and grain mixture plus measured quantities of carotene, codliver oil, or dehydrated alfalfa meal to relieve night blindness; 66 pounds of dried molasses beet pulp, 30 pounds of cottonseed meal, and 4 pounds of decarbonized spent bone black.

Lot 2. Low protein and low phosphorus diet: Cottonseed hulls and grain mixture plus cod-liver oil; 96 pounds of dried molasses beet pulp and 4 pounds of oystershell flour.

Lot 3. Low protein diet: Cottonseed hulls and grain mixture plus cod-liver oil; 96 pounds of dried molasses beet pulp and 4 pounds of decarbonized spent bone black.

The 18 animals in lot 1 were the 9 in individual pens and the 9 kept corralled and fed alfalfa meal as a group for minimum requirements.

[6] COLE, H. H., and MILLER, R. F. CHANGES IN THE REPRODUCTIVE ORGANS OF THE EWE WITH SOME DATA BEARING ON THEIR CONTROL. Amer. Jour. Anat. 57: 39–87, illus. 1935.

They were continued through the breeding and gestation periods on close to minimum levels of vitamin A. When their stores of vitamin A were exhausted and night blindness appeared, vitamin A was supplied in measured quantities as carotene, cod-liver oil, or dehydrated alfalfa meal of known carotene content. Four ewes in lot 1 and eight each in lots 2 and 3 had lambed the previous year.

From June 9, when the experimental ration was started, the cod-liver oil was given to lots 2 and 3 twice weekly by adding 125 cc to the concentrates for each lot on Tuesdays and Fridays. Thus the cod-liver oil was administered at the rate of 2 cc per ewe daily and constituted about twice the minimum requirement.

The total protein content of the ration for lots 2 and 3 was 6.8 percent.

On June 19 all lots were on full feed. The animals in lot 2 were beginning to chew the fences.

On July 3 one ewe in lot 1 died of pneumonia.

On July 30 lots 2 and 3 were sheared, the average weight of wool per head being 3 pounds.

On August 16 the ewes in lot 2 appeared thin and were craving something as was evidenced by their eating dirt and wood. On August 20 lot 1 was in good physical condition, but about half of the animals showed some degree of night blindness.

On August 22 breeding began. Two mature Shropshire rams were alternately used between the three lots during the day and at night were turned out on good pasture.

On September 19 three ewes in lot 2 were especially thin and weak. One of them fell down while being driven a short distance to the scales and had to be carried back to the pen after it was weighed. This animal was 3 pounds heavier than at the previous weighing.

On October 23 breeding seemed to be over. Toward the close of the season a Southdown ram had replaced one of the Shropshires In lot 2 three of the ewes had remained too weak to support a ram had they been in oestrum. On October 31 the rams were taken out after a breeding period of 70 days. Cod-liver oil feeding to lots 2 and 3 was stopped on this date, and these two lots were gradually changed to pasture.

On January 3 one ewe in lot 1 died. She had twin lambs in her uterus and her liver contained 3 units of vitamin A per gram. None of the livers of the lambs born in this group showed any trace of color with the antimony trichloride test. The ewes were slaughtered soon after lambing was completed, and an examination of their livers showed from 8 to 80 units of vitamin A per gram.

Lambing began on January 20. The lambing data are given in table 7, the weights of the ewes in table 8, and the phosphorus determinations on the blood of lots 2 and 3 in table 9.

This fourth year of the experiment was the first in which any of the ewes had been so depleted of vitamin A that symptoms of night blindness were manifested at breeding time. Half of the ewes in lot 1 reached this state of depletion and all had very low reserves. The diet of lot 1, except for the deficiency of vitamin A, was better than that of lots 2 and 3 and the animals consumed more of it. Thus lot 1, which showed symptoms of vitamin A deficiency, still gained 13.1 pounds from August 1 to October 31, and had a lambing record of 76.4 percent. All of the lambs, however, were dead at birth or were

very weak and died within 24 hours. Thus the intake of vitamin A in these ewes was sufficient to prevent them from showing any symptoms more serious than night blindness and yet the level was too low for normal gestation.

TABLE 7.—*Effect of vitamin A depletion and of variations in the vitamin A, phosphorus, and protein content of the ration during breeding and gestation on the lambing record of ewes, 1936*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins [2]	Dry ewes	Total lambs	Lamb crop [3]
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
1	17	9	2	6	13	76.4
2	18	7	2	9	11	61.1
3	18	12	3	3	18	100.0

[1] All of the lambs of lot 1 were born dead or died within 24 hours. In lot 2 a single lamb died at birth posterior presentation requiring assistance. In lot 3 all lambs were alive and normal.
[2] No ewes had triplets.
[3] See footnote 2, table 1.

TABLE 8.—*Effect of vitamin A depletion and of variations in the vitamin A, phosphorus, and protein content of the ration during breeding and gestation on the weight of ewes, 1935*

Date (1935)	Lot no.	Ewes	Average weight	Gain or loss per ewe	Date (1935)	Lot no.	Ewes	Average weight	Gain or loss per ewe
		Number	*Pounds*	*Pounds*			*Number*	*Pounds*	*Pounds*
June 8	1	18	99.4		Sept. 19	1	17	110.4	+0.4
	2	18	103.7			2	18	90.2	+3.0
	3	18	105.1			3	18	103.8	+.9
Aug. 1	1	17	104.5	+5.1	Oct. 23	1	17	116.9	+6.5
	2	18	86.0	−17.7		2	18	84.8	−5.4
	3	18	98.7	−6.4		3	18	97.9	−5.9
Aug. 22	1	17	110.0	+5.5	Oct. 31	1	17	117.6	+.7
	2	18	87.2	+1.2		2	18	86.8	+2.0
	3	18	102.9	+4.2		3	18	98.4	+.5

TABLE 9.—*Effect of vitamin A depletion and of variations in the phosphorus content of the ration during breeding and gestation upon the phosphorus content of the blood serum of ewes, 1935*

[Phosphorus in milligrams per 100 cc of blood serum]

Lot no. and number of ewes	Aug. 4	Sept. 13	Oct. 30	Lot no. and number of ewes	Aug. 4	Sept. 13	Oct. 30
Lot 2:	*Milligrams*	*Milligrams*	*Milligrams*	Lot 3:	*Milligrams*	*Milligrams*	*Milligrams*
116	7.84	10.00		110	8.16	6.28	5.84
117	7.69	7.92	6.61	112	6.89	7.73	7.08
119	6.15	6.53	5.09	114	7.48	6.50	
120	7.34	10.52	9.88	151	8.09		7.08
152	4.85	7.73	8.00	153	11.90	16.00	16.00
154	3.94	4.76	5.55	157	8.42		
155	8.25	8.61	7.08	161	9.88	8.42	8.00
160	7.61	7.69	7.62	165	9.09	8.99	10.25
162	6.56	7.44	5.40	166	10.00	9.82	7.62
164	8.90	10.52	9.20	167	8.00	6.99	5.75
172	7.08	6.45	4.39	173	9.64	8.89	
182	6.30	7.62	6.59	175	8.42	8.70	8.16
183	8.00	6.81	7.28	177	7.14	7.24	6.30
184	8.79	6.93		181	8.90	9.20	8.99
189	7.27	6.45	5.06	187	9.52	10.80	8.00
190	6.25	6.96	5.13	191	8.66	9.25	8.42
192	6.25	7.11	7.62	193	7.92	7.18	4.70
199	8.25	8.08	5.67	197	8.00		6.95
Average	7.07	7.67	6.63	Average	8.67	8.80	7.94

Lot 2, on low phosphorus and protein, lost an average of 17.7 pounds from June 8 to August 1 and from then until the end of the breeding period about maintained their weight. This lot had a lambing record of 61.1 percent. All of the lambs were born healthy except one which died at birth. The appetite of lot 2 was noticeably poor, and depraved appetite was observed during breeding. The number of ewes lambing was 9 out of the 18, and 2 sets of twins were produced. Low phosphorus could not definitely be said to be the controlling factor in this year's studies, for cod-liver oil fed as a vitamin A supplement also contained vitamin D and this may have masked the results. Certainly the physical condition of the sheep in this lot was by far the worst of the three groups. Despite this fact table 9 shows no significant lowering of the blood phosphorus by the regimen to which they were subjected. This may probably be explained by the vitamin D in the cod-liver oil drawing phosphorus stores from the bones of the animals and so maintaining blood phosphorus levels at normal

Lot 3 on low protein but ample phosphorus and vitamin A lost 0.3 of a pound per ewe in weight from August 1 to October 31, and still 15 of the 18 ewes lambed. There were three sets of twins and thus a 100-percent lamb crop was produced. All of the lambs were alive and normal.

The data support the assumption that low protein is not as serious in its effect on fertility as low protein and phosphorus. Animals depleted of vitamin A to the point of partial night blindness will still show oestrum, ovulate, and become pregnant. For normal birth of offspring, however, an ample amount of vitamin A must be supplied during the gestation period.

CONCLUSIONS

The data in 1933 showed that low vitamin A intake in adult ewes over a period of 5 months, including the breeding season, did not affect the number of lambs born as compared to control animals supplied with vitamin A above minimum requirements during the breeding period.

The data in 1934 confirmed the findings of the previous year on vitamin A and also showed that limiting the protein intake over a 4-month period including the breeding season had little effect on the number of lambs produced.

The ewes in these experiments were aged and in good condition with large stores of vitamin A in their livers. The high lambing percentage was not accompanied by sufficient gains in weight of the ewes during the breeding season to constitute the empirical requirements for flushing.

When ewe lambs were used and vitamin A intake restricted, they were still not depleted of this food factor at breeding time, but as the low vitamin A diet was continued during gestation depletion of storage occurred after 9 months and seven of the eight lambs born were weak and died soon after birth.

The fourth year the low vitamin A group was depleted at breeding time to the point of night blindness, but still 11 of the 17 ewes conceived and 2 of these had twins. Gestation was unsuccessful, however, and all of the lambs were born dead or died within 24 hours.

The animals on low protein and phosphorus were more seriously affected by the dietary regimen than animals on low protein alone. Depletion of blood phosphorus did not occur on the low protein and phosphorus regimen; the cod-liver oil may have been responsible for this. Further work will have to be done to establish the effect of low phosphorus.

SELENIUM IN PLANTS IN RELATION TO ITS OCCURRENCE IN SOILS[1]

By JOHN T. MILLER, *assistant soil surveyor, Soil Survey Division*, and HORACE G. BYERS, *principal chemist, Soil Chemistry and Physics Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

In studying the distribution and intensity of selenium in soils, the writers have made certain observations which it is believed will be of interest to botanists in general and to plant ecologists in particular.

From the studies which followed the recognition of selenium in plants as the cause of a characteristic disease of animals, certain plant relationships have gradually emerged.[2] It first appeared that all plants grown on seleniferous soils absorb selenium to some extent. Later it became apparent that certain plants are particularly prone to this absorption and thereby become intensely toxic (fig. 1). Thus, a sample of *Astragalus bisulcatus* (Hook.) A. Gray from Albany County, Wyo., contained 4,300 parts per million of selenium[2]; a sample of *A. racemosus* Pursh from Dawes County, Nebr., contained 5,560 parts per million; and a sample of *A. pectinatus* (Hook.) Dougl. from Logan County, Kans., contained 1,750 parts per million. A sample of *Stanleya pinnata* (Pursh) Britt. from Otero County, Colo., contained 1,880 parts per million, and a sample of *Applopappus fremonti* A. Gray from Crowley County, Colo., contained 760 parts per million. Such quantities of selenium are lethal when ingested by animals.

On the other hand, many plants on the same areas show no such absorptive capacity. In exactly the same soil area a sample from a plant of *Astragalus bisulcatus* growing among wheat plants contained 1,110 parts per million of selenium while the wheat contained but 45. *A. missouriensis* Nutt. contained but 3 parts per million,[2] where *A. bisulcatus* contained 1,250 parts per million. Native prairie grasses for the most part seem to have a very low absorptive capacity, as illustrated by the fact that on a single square mile in South Dakota where 15 samples of wheat contained an average quantity of 21 parts per million the average for 50 samples of little bluestem was but 0.8. No samples of buffalo grass, of hundreds examined, have been found to contain toxic quantities of selenium.

As pointed out in a recent publication,[3] there appears to be no direct quantitative relationship between the selenium present in a soil and that absorbed by the plants, even of a given species. One reason for this appears to be the variation in the forms of selenium present in soils.[4] Nevertheless, where the soils have a relatively high selenium content, plants in general show increased quantities of selenium.

[1] Received for publication Nov. 27, 1936; issued August 1937.

[2] BYERS, H. G. SELENIUM OCCURRENCE IN CERTAIN SOILS IN THE UNITED STATES WITH A DISCUSSION OF RELATED TOPICS. U. S. Dept. Agr. Tech. Bull. 482, 48 pp., illus. 1935.

[3] BYERS, H. G. SELENIUM OCCURRENCE IN CERTAIN SOILS OF THE UNITED STATES WITH A DISCUSSION OF RELATED TOPICS. SECOND REPORT. U. S. Dept. Agr. Tech. Bull. 530, 79 pp., illus. 1936.

[4] WILLIAMS, K. T., and BYERS, H. G. SELENIUM COMPOUNDS IN SOILS. Indus. and Engin. Chem. 28: 912-914. 1936.

FIGURE 1.—*Astragalus bisulcatus* growing on soil developed from Pierre shale, Fall River County, S. Dak., June 10, 1935.

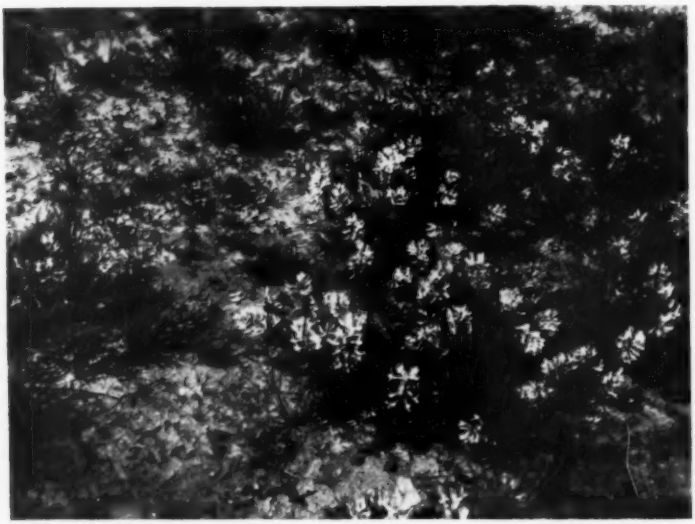

FIGURE 2.—*Astragalus pectinatus* growing on soil developed from Pierre shale, Shannon County, S. Dak., June 7, 1935.

Even before the recognition of selenium as a cause of the so-called alkali disease an effort was made to relate the incidence of the disease to the soil,[5] and it was shown that the areas best known to produce

[5] FRANKE, K., RICE, T. D., JOHNSON, A. G., and SCHOENING, H. W. REPORT ON A PRELIMINARY FIELD SURVEY OF THE SO-CALLED "ALKALI" DISEASE OF LIVESTOCK. U. S. Dept. Agr. Circ. 320, 10 pp., illus. 1934.

toxic grain were on soils derived from Pierre shales in South Dakota and Nebraska.[5] It was correctly assumed that the source of the injury was in the shales. After the occurrence of selenium in these shales had been established, it was found that selenium was also present in the Niobrara shales and in soils developed from them. These shales, like the Pierre, are a part of the formations of the Upper Cretaceous period. It appears probable that other formations of the Cretaceous period are abnormal with respect to selenium content, and possibly certain younger formations as well.

In the spring of 1935, during the course of a reconnaissance survey of possible seleniferous areas, the writers were impressed by the fact that almost invariably where Niobrara and Pierre formations (fig. 2) were the probable sources of soils, one or more species of *Astragalus* or *Stanleya* were found. In many cases in South Dakota, Nebraska, and Wyoming arrival on these soils was as readily recognized through observation of the botanical associations as through a study of the soils or of the geologic maps. Therefore, during a somewhat detailed study of certain areas in western Kansas, observations were made as outlined below. These observations were further supplemented by observations in Colorado and New Mexico.

OBSERVATIONS IN KANSAS

In Kansas the area of study was confined to the upper drainage basins of the Smoky Hill, Saline, and Solomon Rivers, where these streams cut through the loessial mantle into the Pierre and the soft chalky shales of the upper Niobrara formations (Smoky Hill). The physiography of this region reduced to its simplest terms is that of a plain, slightly tilted toward the east. It is characterized by broad level interstream areas, and short, moderately steep slopes bordering the valleys of streams. Except for restricted areas, the surface of this plain is covered by a thick mantle of loess, which in the northwestern corner of the State attains a thickness of 180 feet. Consequently, the soils over the greater portion of the area are developed from materials of loessial origin. Soils developed from residual materials are confined for the most part to those on slopes bordering streams or in close proximity to streams where the loessial mantle has been removed by erosion, or in first bottoms or terraces where the parent materials are largely residual.

Keith silt loam, a Chestnut soil, is the dominant soil of the uplands. In this soil type the upper 15 inches is dark grayish brown and the upper 3 inches is slightly laminated. At a depth of 15 to 30 inches, the soil consists of a slightly compacted heavy silt loam with a slightly prismatic structure which breaks into clods. This is underlain to depths of 10 feet or more by light-brown silty loess (Sanborn loess). The profile has a heavy accumulation of carbonates at a depth of 20 to 35 inches. The normal vegetative cover where the Keith silt loam is fully developed consists of a dense sod of buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.) and blue grama (*Bouteloua gracilis* (H. B. K.) Lag.) with lesser amounts of side-oats grama (*B. curtipendula* (Michx.) Torr.). Along with these dominates, occasional plants of a large number of species are scattered over the terrain. These miscellaneous plants increase in number where the range is overgrazed in relation to the grass cover. On this soil type the species

[5] See opposite page for footnote.

of *Astragalus* mentioned above seldom appear even when they are of frequent occurrence on neighboring areas.

At a typical location of Keith silt loam in the SW¼ sec. 7, T. 11 S., R. 28 W., the following cover was observed:[6]

Buffalo and grama grasses, 75 percent.
Miscellaneous vegetation, 25 percent.

The miscellaneous cover consisted of:

Sunflower (*Helianthus annuus* L.).
Russian-thistle (*Salsola pestifer* A. Nels.).
Psoralea (*Psoralea collina* Rydb.).
Sagebrush (*Artemisia Frigida* Willd.).
White wreath aster (*Aster ericoides* L.) (*A. multiflorus* Ait.).
Kuhnia (*Kuhnia glutinosa* Ell.).
Common pigweed (*Amaranthus* sp.).
Blazing-star (*Laciniaria punctata* Hook.).

The surface soil at this point contained but 0.4 part per million of selenium, and the wild aster, which is the best selenium absorbent of this group of plants, contained but 2 parts per million. The other forms of vegetation at this point were not collected for analysis.

Three typical soil profiles, the environments of which differ chiefly in topography, are developed on Smoky Hill shale (upper Niobrara). They are as follows:

(1) On smooth upland: The upper 8 inches consists of dark grayish-brown silt loam. This merges into a brown light silty clay loam which is underlain at 12 inches by a heavy silty clay loam. At a depth of about 35 inches the soil merges into yellowish-brown lighter textured silty clay loam, which in turn merges into the dull-brown Smoky Hill clay shale. The entire depth of this type of profile from surface to shale is 40 to 60 inches. The whole profile is calcareous, with a heavy carbonate accumulation at about 25 to 40 inches.

(2) On moderately steep slopes: The soil profile is similar to that developed on the smooth upland except that both the surface soil and subsoil horizons are shallow and the transitional zone from soil to shale is strongly modified or absent. The unweathered shale occurs within 20 inches of the surface. In places only 1 or 2 inches of grayish-brown topsoil is developed.

(3) On nearly level local alluvial areas: At the base of outcrops of Smoky Hill shale, such as those in the vicinity of the "Pyramids" of Gove County (fig. 3), the soil consists of a yellowish-brown chalky light clay loam which may extend to a depth of 4 feet or more. It is usually undifferentiated, but in places where the accumulation of shale materials is slow, 6 to 8 inches of grayish-brown surface soil has developed.

On all the modifications of the soil developed from the Smoky Hill materials the normal vegetative cover is sparse. In its extreme expression it consists of turpentine weed (*Gutierrezia sarothrae* (Pursh) Britton & Rusby), constituting a 25-percent cover, and a scattering of other plants, making a maximum cover of 35 to 40 percent. In places rabbitbrush (*Chrysothamnus* sp.) is a common plant associated with the turpentine weed. In a few locations *Eriogonum* sp. is common. One of these locations is in the NE¼ sec. 30, T. 13 S.,

[6] Valuable assistance in the identification of plants for this paper was rendered by S. F. Blake of the Bureau of Plant Industry, United States Department of Agriculture, and by E. C. Smith of the Colorado State College of Agriculture, Fort Collins, Col.

R. 35 W., in Logan County. *Mentzelia decapetala* (Pursh) Urban & Gilg., *Aster ericoides* (white wreath aster), *A. fendleri* A. Gray (blue aster), and *Tetraneuris fastigiata* Greene are plants of minor importance on shaly slopes and immediately adjacent areas.

On the soils developed from local alluvium and colluvium adjacent to steep slopes there is a more profuse growth of vegetation. On these areas, however, the vegetative cover is sparse as compared to the normal cover on the Keith silt loam. One of the dominant plants on these soils is turpentine weed, which constitutes from 5 to 25 percent of the cover. At many locations there is a straggly growth of wild aster, interspersed with side-oats grama. In other places one or the other or both of the wild asters mentioned above may constitute a 5- to 10-percent cover. This is especially true on narrow, alluvial

FIGURE 3.—View showing sparse vegetation cover on local alluvium surrounding the "Pyramids," Gove County, Kans. The vegetation in the foreground consists largely of turpentine weed (*Gutierrezia sarothrae*). August 13, 1935.

areas. Little bluestem (*Andropogon scoparius* Michx.), *Muhlenbergia* sp., and *Aristida* sp. are grasses of minor importance. Other plants of minor importance are *Mentzelia decapetala*, *M. nuda* (Pursh) Torr. & Gr., *Psoralea tenuiflora* Pursh, *P. digitata* Nutt., *Lygodesmia juncea* (Pursh) D. Don, Russian-thistle, and sunflower.

At a typical location on a terrace where the soil was derived largely from Smoky Hill materials, an area 50 feet square, 150 feet from the northeast corner of sec. 30, T. 13 S., R. 35 W., in Logan County, was examined in detail. This area was approximately 80 rods from the nearest shale outcrop. The data obtained are as follows: Buffalo grass, 15-percent cover; *Muhlenbergia* sp., 5 percent; *Aristida* sp., 1 percent; a few scattering plants of side-oats grama, 5 plants of *Astragalus pectinatus*, 25 of *Stanleya bipinnata*, Greene, 11 of *Gutierrezia sarothrae*, 2 of *Cirsium lanceolatum* (L.) Hill, 1 of yucca, and 5 of *Helianthus annuus*. There were numerous *Salsola pestifer* plants,

and approximately 1 *Malvastrum coccineum* (Pursh) Gray for each 2 square feet. The total cover was estimated at 40 percent. At this location the surface soil contained 22 parts per million of selenium, the *A. pectinatus* 500 parts per million, based on the air-dry plant, and the *Aristida* 5 parts. The other plants were not examined for selenium.

On another area 50 feet square, located 80 rods south of the one just described, and consisting in part of shaly outcrop and in part of chalky materials of local alluvial and colluvial origin, the cover was as follows: On the shaly outcrop, 20 plants of *Eriogonum* sp. and 1 plant of *Yucca* sp.; on the chalky colluvial material the cover, about 30 percent, consisted of turpentine weed 15 percent, and Russian-thistle 10 percent, along with 20 plants of wreath aster, 5 of blue aster, 10 of *Stanleya pinnata* (Pursh) Britt., and 1 *Psoralea* (*P. digitata*). While these samples were not examined for selenium, the plant association is typical of proven seleniferous soils in this area, and occurring as it does on a shaly outcrop, would justify the assumption that they were seleniferous.

OBSERVATIONS IN COLORADO AND NEW MEXICO

In the spring of 1936 the writers were engaged upon a second scouting expedition through eastern Colorado, New Mexico, Arizona, and Utah, where, on geological and other grounds, the existence of seleniferous areas was suspected. It was observed that wherever the soils were derived from shales corresponding to the Pierre or Niobrara formations, one or more of the plant species which had been found to be good selenium absorbers were always present. A few examples may be cited. In Hamilton County, Kans., no specimens of *Astragalus racemosus* were observed until a small outcrop of soil derived from Niobrara shale, in sec. 2, T. 22 N., R. 43 E., was reached. Here, in a soil sample containing 1.5 parts per million of selenium, and over shale containing 8 parts per million, a plant of *A. racemosus* was growing. It was examined for selenium and found to contain 1,220 parts per million.

In various locations in the Arkansas Valley between Holly and Pueblo, Colo., numerous samples of *Astragalus bisulcatus*, *A. racemosus*, and *A. pectinatus* were collected, and invariably these plants were found to contain selenium in quantities varying from 5 to several thousand parts per million. On United States Route No. 85, from Raton to Las Vegas, N. Mex., wherever outcrops of Pierre and Niobrara shales were found as soil parent materials, various species of *Astragalus* were observed. Five miles from Raton a specimen of *A. racemosus* was collected which contained 390 parts per million of selenium, and the surface soil in which it was growing had 2.5 parts. At a point 14 miles from Raton a sample of *A. bisulcatus* growing on a clay loam which, at the surface, contained 12 parts per million of selenium, was found to contain 2,580 parts. Again, where the route passed over volcanic formations and various other strata, no species of *Astragalus* were observed. Within a few miles of Las Vegas a species of *Astragalus*, tentatively identified as *A. bisulcatus*, appeared. It contained 1,110 parts per million of selenium, and the surface soil in which it was growing contained 1.5 parts. On the highway between Shiprock, N. Mex., and Cortez, Colo., *Stanleya pinnata* (fig. 4) was observed only where the soils were seleniferous. Here samples

were found containing from 70 to 830 parts per million of selenium. In traversing the route from Glenwood Springs, Colo., to Colorado Springs on United States 40 S., no known seleniferous shales outcrop until Walcott is reached. No species of *Astragalus* were noted along this route. North of Walcott the geological map shows a small outcrop of Niobrara shale. A short inspection of this area along State Road No. 11 revealed the presence of numerous specimens of *A. racemosus*. A single plant examined for selenium was found to contain 2,820 parts per million. The upper 6 inches of soil upon which this plant was growing contained 6 parts per million of selenium.

FIGURE 4.—*Stanleya pinnata* plant growing on soil developed from Apishapa shale, Otero County, Colo. August 1, 1936. Note lack of grass cover.

To determine more definitely the relationships indicated by the foregoing data two closely adjacent spots in Kiowa County, Colo., were selected. One of these is a soil area developed over Apishapa [7] (Niobrara) shale and undoubtedly is seleniferous, as indicated in the tabular data. It is located 80 rods north of the southwest corner of sec. 6, T. 19 S., R. 51 W. The other soil is developed over the Ogallala formation (a slightly consolidated sandstone of Tertiary age) and is as free from selenium as can be expected in this area. It is located near the center of sec. 23, T. 18 S., R. 51 W. Both areas are topographically similar and are smooth and uniform in character. At each point an area 100 feet square was selected and the vegetation studied and sampled. At each plot a profile of soil was taken. All the samples obtained were examined for selenium. The results are given in table 1.

The seleniferous area examined was part of a virgin area which probably has never been overgrazed. The cover was estimated: Blue grama, 20 percent; turpentine weed, 10 percent; spurge, 3 percent; miscellaneous plants, 17 percent; total cover, 50 percent. The

[7] In Colorado the upper Niobrara is called Apishapa shale, whereas in Kansas this part of the Niobrara is called Smoky Hill shale.

miscellaneous vegetation included 1 *Astragalus pectinatus*, 10 *Stanleya pinnata*, and 12 *Applopappus fremonti* plants.

The nonseleniferous plot selected was part of an area which had been previously overgrazed. This was selected in order to obtain a variety of vegetation. The normal vegetation of the soil developed on the Ogallala formation consists of a dense cover of grasses, chiefly buffalo and grama grasses. The area selected had an estimated total cover of 75 percent, 50 percent of buffalo and grama grasses, 15 percent of Russian-thistle, and 10 percent of miscellaneous vegetation.

TABLE 1.—*Selenium content of soil profiles and vegetation on a "nonseleniferous" and on a "seleniferous" area* [1]

Area and laboratory sample no.	Field no.	Material	Selenium in—	
			Soil	Vegetation
Nonseleniferous: [2]			*P. p. m.*	*P. p. m.*
B19510	62	Heavy silt loam, 0–6 inches	0.2	
B19511	62	Gritty clay loam, 6–12 inches	.2	
B19512	62	Stiff clay loam, 12–24 inches	.2	
B19513	62	Clay loam, 24–36 inches	.2	
B19514	62	Silt loam, 36–48 inches	.2	
B19515	62	Sandy loam	.2	
B19516	62a	Blue grama (*Bouteloua gracilis*)		0
B19517	62b	Russian-thistle (*Salsola pestifer*)		1
B19518	62c	Scarlet mallow (*Malvastrum coccineum*)		1
B19519	62d	Silky sophora (*Sophora sericea* Nutt.)		1
B19520	62e	Sunflower (*Helianthus annuus*)		1
B19521	62f	Corn [3] (*Zea mays* L.)		1
B19522	62g	Sagebrush (*Artemisia frigida*)		1
B19523	62h	Cocklebur (*Xanthium* sp.)		2
B19524	62i	Spurge (*Euphorbia* sp.)		0
B19525	62j	False buffalo grass (*Munroa squarrosa* Torr.)		1
Seleniferous: [4]				
B19526	63	Gritty clay loam, 0–6 inches	2.0	
B19527	63	Gritty clay loam, 6–12 inches	3.5	
B19528	63	Silty clay loam, 12–24 inches	4.0	
B19529	63	Shaly clay loam, 24–36 inches	4.0	
B19530	63	Apishapa shale, 36–48 inches	3.5	
B19531	63a	Blue grama		2
B19532	63b	Russian-thistle		5
B19533	63c	Scarlet mallow		1
B19534	63d	(*Oreocarya* sp.)		1
B19535	63e	Sunflower		2
B19536	63f	Corn [5]		10
B19537	63g	Stanleya (*Stanleya pinnata*)		330
B19538	63h	Cocklebur		6
B19539	63i	Spurge		10
B19540	63j	False buffalo grass		4
B19541	63k	Turpentine weed (*Gutierrezia sarothrae*)		70
B19542	63l	Applopappus (*Applopappus fremonti*)		320
B19543	63m	Narrow-leaved milkvetch (*Astragalus pectinatus*)		4,000

[1] All analyses by K. T. Williams and H. W. Lakin, Bureau of Chemistry and Soils, U. S. Department of Agriculture.
[2] Fields located in Kiowa County, Colo., near center sec. 23, T. 18 S., R. 51 W.
[3] From cultivated field at edge of plot growing under similar soil conditions.
[4] Fields located in Kiowa County, Colo., 80 rods north of southwest corner sec. 8, T. 19 S., R. 51 W.
[5] Volunteer plant at edge of plot growing under similar soil conditions.

CONCLUSIONS

From observations made over wide areas where seleniferous soils occur in juxtaposition to soils relatively free from selenium, and from the examination of several thousand plant samples for selenium, it is believed that plants may be classified in three broad groups in relation to their tolerance of selenium in the soil. Incidentally, it may be remarked that hydrogen-ion concentration does not appear to be an important influence in relation to selenium absorption. The soils

here specifically mentioned all have a pH value in excess of 7, the range being 7.5 to 8.4.

(1) Plants that absorb selenium readily: Among these are *Astragalus racemosus* (fig. 5). *A. pectinatus, A. bisulcatus, A. carolinianus* L, *Stanleya pinnata, S. bipinnata, Applopappus fremonti, Xylorrhiza parryi* (A. Gray) Greene, and probably other species of the same and different genera. It is not to be inferred that all species of a given genus possess like properties in this respect, since some of the species of *Astragalus*, notably *A. missouriensis, A. mollissimus*, and *A. drummondii* Dougl., have been found to contain very small quantities of selenium even when growing on very seleniferous soil. In almost every location

FIGURE 5.—*Astragalus racemosus* root system developed in Apishapa shale. Note limonite and marcasite concretion at location of penknife and small feeder roots of the *Astragalus* at this place in the shale. Pueblo County, Colo., Aug. 29, 1936.

where these highly selenized plants have been found on seleniferous soil, they have been wholly absent or very rare on adjacent nonseleniferous areas. Since analyses show these plants to be consistently high in selenium and to be growing vigorously even with a very high content, it seems possible that selenium may be of importance to their physiological processes. This suggestion is emphasized by the observed fact that in Trego and Ellis Counties, Kans., the three species of *Astragalus* mentioned were found well distributed over the soil areas derived from Smoky Hill shale but were not observed beyond the eastern limit of such soil.

(2) Plants that are able to absorb moderate, or even large, quantities of selenium without severe injury: Such plants are found widely distributed on both seleniferous and nonseleniferous areas. Among such plants are white wreath aster, blue aster, turpentine weed, sunflower, western wheatgrass (*Agropyron smithii* Ryab.), and perhaps others. Included in this group are the common cereals,

wheat, rye, barley, and corn. To what extent the growth of such plants is retarded by the presence of small quantities of selenium is not known, but it is certain that there is a rather low tolerance limit for the soil.

(3) Plants that have a very limited tolerance of selenium and are able to absorb only small quantities when grown on seleniferous soils: In places where the concentration of selenium in the surface soils is high these plants either do not appear or they are of limited occurrence and make poor growth. The most notable examples of this group are buffalo grass and the grama grasses *Bouteloua gracilis* and *B. curtipendula*.

THE SUSCEPTIBILITY OF POTATOES TO THE VEIN-BANDING VIRUS[1]

By LEON K. JONES, *associate plant pathologist*, and C. L. VINCENT, *associate horticulturist, Washington Agricultural Experimeut Station*

INTRODUCTION

Tests in connection with tuber indexing of commercial lots of seed potatoes (*Solanum tuberosum* L.) in 1929–32 showed that the vein-banding virus was responsible for a large amount of loss to potato growers in the State of Washington. Since this virus spread rapidly in field plantings in the vicinity of Pullman, an opportunity was afforded to study (1) its rate of spread, (2) its effect upon varieties and seedling clones, and (3) the degree of resistance of varieties and seedling clones to infection under field conditions.

Numerous tests with the vein-banding virus discussed in this paper have shown it to have the following characters: Remains active in vitro at room temperature for 4 to 6 days and in dried plant tissue 17 to 50 days (*1*)[2]; inactivated in vitro at 55° C. for 10 minutes; produces spot necrosis of tobacco and tomato plants when used as inoculum in combination with the potato latent virus; is transmitted by aphids (*Myzus persicae* Sulz.); does not infect *Datura stramonium* L.; and does not modify the expression of tobacco mosaic on tobacco or tomato plants. This virus evidently is a variant of the Y virus (*3*, *10*) and corresponds to potato virus 20 in the tentative classification of viruses by the international committee.[3]

The term latent virus as used in this paper refers to the mild latent form (*6*), a variant of the X virus (*10*) and possibly the same as potato virus 16.[3]

The terms used in the text and in the tables to denote symptoms that were observed in the plants infected by viruses may be defined as follows:

Crinkling.—Slight, wavy unevenness of the leaf blade as contrasted with the smooth appearance of healthy foliage.

Curly dwarf.—Pronounced dwarfing, rugosity, curling, brittleness, and acropetal necrosis of leaves and stems (fig. 1).

Mottling.—Spotting of leaf blades by light green areas which vary in size, shape and degree of paleness.

Necrosis.—Premature death of tissues resulting in a brown discoloration.

Rugose mosaic.—Dwarfing, diffuse mottling, rugosity, necrotic spotting and acropetal dropping of foliage.

Spot necrosis.—Somewhat irregular, necrotic spots and chlorotic lines in the foliage, preceded by vein clearing and rugosity in tobacco leaves, caused by the combined action of the latent and the vein-banding viruses (*6*).

Streak.—Brown to black necrotic areas in leaves and stems of tomato plants, caused by the combination of the potato latent virus and the tobacco mosaic virus (*2*).

Tobacco mosaic.—Mottling of tobacco or tomato foliage caused by the tobacco mosaic virus 1 (*4*).

[1] Received for publication Nov. 30, 1936; issued August 1937. Published as Scientific Paper no. 356, College of Agriculture and Experiment Station, State College of Washington.

[2] Reference is made by number (italic) to Literature Cited, p. 79.

[3] INTERNATIONAL COMMITTEE ON DESCRIPTION AND NOMENCLATURE OF PLANT VIRUSES. ILLUSTRATION OF PROPOSED SYSTEM OF NOMENCLATURE FOR PLANT VIRUSES. 1936. [Mimeographed report.]

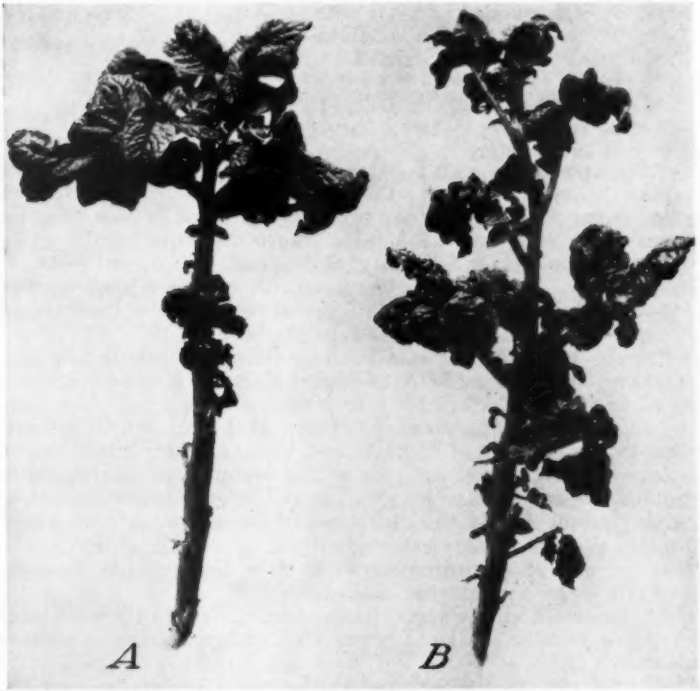

FIGURE 1.—Curly dwarf symptoms produced in potato plants by the vein-banding virus: *A*, Chippewa; *B*, Katahdin.

Top necrosis.—Necrotic spotting and premature death of upper portions of the plant, progressing downward.

Vein banding.—Slight mottling of tobacco leaves by interveinal light green areas in contrast to the normal green adjacent to the veins (*2*).

METHODS

Varieties and seedling clones of potatoes were planted in the field near Pullman each summer from 1932 to 1936, inclusive. At least two field observations were made each season to note the symptoms and the rate of spread of viruses. The viruses occurring in the field plantings have been identified by tests in the greenhouse during the winter months each year. The earlier tests were made with 1 tuber from each hill but in the later tests 5 to 20 tubers from each variety or clone were indexed.

The index potato plants were tested in the greenhouse by inoculating from each potato plant into six tobacco plants as follows: Two with macerated potato-leaf tissue alone; two with macerated potato-leaf tissue plus macerated tobacco tissue infected by the latent virus; and two with macerated potato-leaf tissue plus macerated tobacco tissue infected by the vein-banding virus. Since the latent and vein-banding viruses together produce distinctive symptoms of spot necrosis when incoluated into tobacco plants, the presence of the

vein-banding virus or the latent virus in the potato plants was readily determined.

All inoculations into plants were made with macerated plant tissue according to the method described by Jones (*5*) with the use of carborundum as described by Rawlins and Tompkins (*7*).

FIELD TESTS WITH THE PROGENY OF A VIRUS-FREE EARLY ROSE TUBER

During the winter of 1929, when indexing commercial stock, a single tuber of the Early Rose variety was found to be free from both the latent and the vein-banding viruses (*2*). Virus-free stock from this tuber was developed under cloth chambers in the greenhouse during the winter months of 1929–31 until 142 virus-free tubers were available. This stock was planted in the field in 1932, and its progeny was grown in the field in 1933 and 1934. All plantings were made in

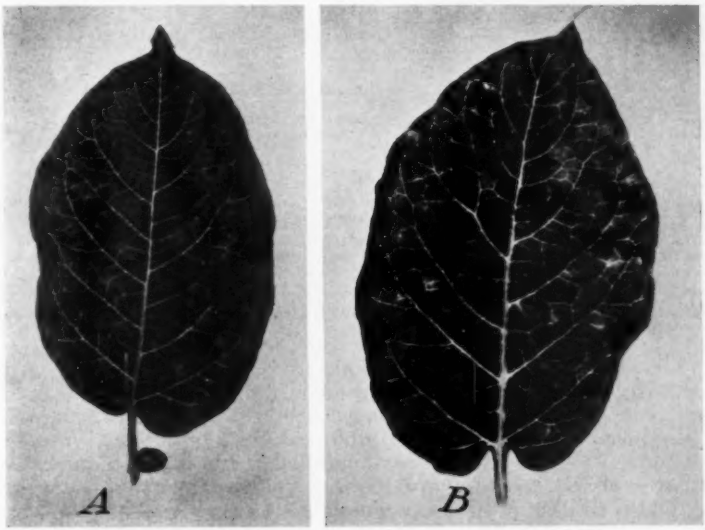

FIGURE 2.—Mottling of potato leaflets caused by the vein-banding virus: *A*, Early Rose; *B*, Katahdin.

locations at least one-half mile from other potatoes, except that virus-free seedlings were included in the planting in 1933.

Each winter one tuber from each hill produced the previous season was indexed in the greenhouse. The nature of the virus found in the plants was studied by inoculations from each potato plant into tobacco plants, according to the method described above.

The results of these tests show that the 142 hills grown in 1932 remained virus-free; the 314 hills grown in 1933 became 59 percent infected; and the 840 hills grown in 1934 became 100 percent infected with the vein-banding virus during the growing season. No other virus was noted in the Early Rose stock. The symptoms produced by the vein-banding virus were similar to those associated with mild mosaic, which included slight dwarfing of the plants and mild mottling and crinkling of the foliage (fig. 2, *A*).

In December 1932, following the harvest of the 142 hills one tuber from each hill was planted in the greenhouse for indexing. After the index tests with tobacco and tomato plants had shown all plants to be free from viruses the plants were grouped in series of four plants each, separated from one another by cloth partitions. The first group of four plants was then inoculated with the latent virus; the second with the vein-banding virus; the third with the combination of these two viruses; and the fourth was uninoculated. Six weeks later inoculations were made from portions of each potato plant into tobacco and tomato plants. A summary of the results of these tests is shown in table 1.

TABLE 1.—*Results of virus inoculations into virus-free Early Rose potato plants,*[1] *Pullman, Wash., 1933*

Inoculum from tobacco plants	Symptoms produced in potato plants	Symptoms produced by inoculations from infected potato foliage in—				
		Tobacco plants			Tomato plants	
		Potato inoculum alone	Potato inoculum plus latent virus	Potato inoculum plus vein-banding virus	Potato inoculum alone	Potato inoculum plus tobacco mosaic virus
Vein-banding	Crinkling, mottling.	Vein banding.	Spot necrosis.	Vein banding.	Vein banding	Tobacco mosaic.
Vein-banding plus latent.	Rugose mosaic.	Spot necrosis.	do	Spot necrosis.	Spot necrosis.	Streak.
Latent	Crinkling	Mottling	Mottling	do	Mottling	Do.
Control	None	None	do	Veinbanding	None	Tobacco mosaic.

[1] The Early Rose plants were found to be virus-free by inoculations from the foliage of each potato plant into tobacco plants; 4 plants used in each test.

The vein-banding virus, mechanically inoculated into the virus-free Early Rose stock, produced a definite mottling and crinkling of the foliage similar to the symptoms noted in plants naturally infected in the field. The latent virus produced slight crinkling symptoms without mottling, while the combination of vein-banding and latent viruses produced mottling and rugosity of the foliage and necrosis of the lower leaves, symptoms commonly associated with the term "rugose mosaic."

TESTS WITH SEEDLING CLONES

SEED FURNISHED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE

Thirty-one seedling clones, having desirable plant growth, tuber yield, and shape were increased in the greenhouse during the winters of 1931 and 1932 from the seeds of potato (no. x325: Green Mountain × Katahdin) furnished in 1929 by C. F. Clark of the United States Department of Agriculture. Index tests with this stock in the greenhouse during the spring of 1933 showed no viruses present in the tubers. Two to thirteen hills of each virus-free clone were planted in the field along with the virus-free Early Rose stock in 1933. Indexing of one tuber from each hill during the winter of 1933–34 showed that plants of the seedling clones became infected by the vein-banding

virus in the field as follows: 13 clones, 100 percent; 6 clones, over 50 percent; 5 clones, 10 to 50 percent; 7 clones virus-free. The results of these tests indicate that the seedling clones differed in susceptibility to infection by the vein-banding virus.

LOCALLY DEVELOPED SEED

A large number of seedling clones of potatoes have been developed by the junior author, and the more promising ones have been grown on the farm of the State College of Washington for 2 to 13 years. A

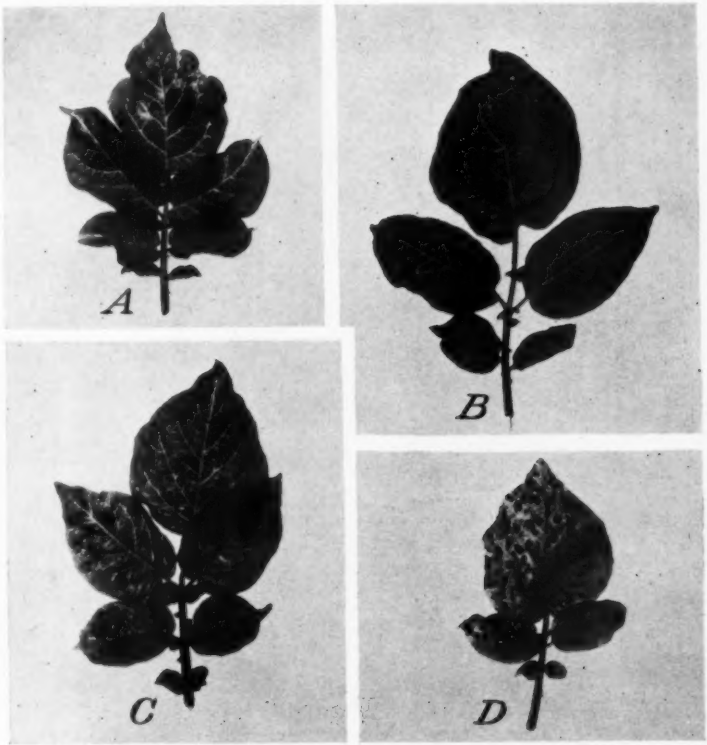

FIGURE 3.—Mottling and necrosis produced in seedling potato leaves by the vein-banding virus: *A*, Seedling 1165-7; *B*, seedling 1152-19; *C*, seedling 1358; *D*, seedling 1045-3.

row of potatoes infected by the vein-banding, latent, and leaf-roll viruses was planted every third to fifth row, throughout the field each season. Although the seedlings were not uniformly exposed to infection by these viruses ample opportunity for infection was afforded; therefore, large and consistent differences in the percent of disease noted in the clones should be significant.

Field examinations of the seedlings were made during 1935–36 and the prevalence of viruses and attendant symptoms was recorded. All of the virus-free clones and those recorded as "virus infection question-

able" from the field observations as well as many of the virus-infected clones were indexed each winter. Five to twenty tubers representing each clone to be tested were planted in the greenhouse and the indexing consisted in observing the symptoms of disease, if any, shown by each plant and inoculating with macerated leaf tissue from each potato plant into tobacco plants according to the method described above.

TABLE 2.—*Results of tests with seedling strains of potatoes to determine susceptibility to infection by the vein-banding virus*

Seedling no. [1]	Parentage	Clones observed	Years grown in field	Clones virus-free [2]	Clones showing symptoms of vein-banding	
					Mottling	Necrosis
		Number	*Number*	*Number*	*Number*	*Number*
50	Early Six Weeks × McCormick	2	12	0	2	
54	Late Rose × Bovee	1	11	0	1	
194–208	Early Northern × McCormick	4	9	0	4	
1028–1064	Keeper, selfed	10	7	0	9	1
1065–1067	Jersey Red Skin × Keeper	10	7	0	10	
1068	Keeper × McCormick	1	7	0	1	
1069	216–3 × 205–2	3	7	0	3	
1145–1148	McCormick × Keeper	8	7	0	7	4
1152–1154	Keeper × 198–13	3	7	0	3	
1157–1169	Irish Cobbler × Keeper	22	6	0	22	
1171–1180	American Wonder × Keeper	22	6	0	20	2
1181	1026, selfed	2	6	0	2	
1203–1358	Russet Burbank, selfed	6	6	0	6	
1425–1426	American Wonder × Keeper	7	6	0	7	2
1582	Irish Cobbler, selfed	1	6	0	1	
1611–1736	do	3	5	0	2	1
1807–1953	Katahdin, selfed	73	4	2	62	11
1954–2082	205–2, selfed	64	4	0	64	1
2103–2150	Irish Cobbler, selfed	3	4	0	3	
2159–2382	194–10, selfed	97	4	0	97	
2384–2417	57–1, selfed	26	4	0	26	
2418–2451	198–2, selfed	21	4	0	21	
2518–2615	Katahdin, selfed	97	2	5	83	9
2516–2630	205–5 × 208–5, selfed	15	2	0	15	
2631–2646	1147–2 × 1046–3, selfed	16	2	0	16	
2647–2745	Katahdin, selfed	99	2	8	75	16
2746–2756	1047–2 × Katahdin	11	2	0	9	2
2757–2842	1321, selfed	86	2	0	86	
2843–2878	1148–1 × 1155–14, selfed	36	2	0	33	3
2879–2902	1157–13 × Katahdin	24	2	1	13	11
2903–2949	1142–1 × 1155–14	47	2	0	43	4
2950	200–5, selfed	1	2	0	1	
2951–2953	1163–1 × 1069–4	3	2	0	3	
2954–2956	Katahdin × Eureka	3	2	0	3	
2957–2973	1148–1 × 1065–3	16	2	0	15	1
2974–3011	Katahdin × 1185	38	2	5	18	15
3012–3026	1159–13 × Katahdin	14	2	0	9	5
3027–3091	205–2, selfed	65	2	0	65	
3092–3099	Katahdin × 1425–9	8	2	0	2	6
3100–3105	Katahdin × 1154–13	6	2	0	4	2
3106–3113	1175–15 × Katahdin	8	2	0	7	1
3114–3127	1148–1 × 1025–2	14	2	0	14	
3128–3133	Katahdin × 1154–3	6	2	0	4	2
3134–3168	200–5 × Katahdin	35	2	1	28	6
3169–3176	Katahdin × 1321	6	2	1	2	3
3177–3182	Katahdin × 1328	6	2	1	3	2
3183–4293	Katahdin, selfed	1,096	2	56	922	118
4294–4326	205–2 × Katahdin	33	2	5	23	5
4327–4375	205–2, selfed	49	2	0	49	
4376–4425	1157–6, selfed	50	2	0	47	3
4426–4509	205–2, selfed	84	2	0	83	1
4510–4542	198–2, selfed	33	2	0	33	
4543–4558	1033–4, selfed	15	2	0	15	
4559–4590	198–2, selfed	32	2	0	31	1
4591–4599	Katahdin, selfed	9	2	4	2	3

[1] Records of seedling clones 50 to 3182, inclusive, were made in 1935. Records of clones 3183–4599, inclusive, were made in 1936.

[2] Determined by field observations on 50 or more hills in each clone, and by greenhouse tests with all clones recorded as virus-free and with many of the clones recorded as virus-infected.

Results of the tests with seedling clones developed from crossed and selfed varieties of potatoes showed that Katahdin was the only parent that transmitted resistance to infection by the vein-banding virus (table 2). Of the 1,572 clones, 89 (5.7 percent) developed from Katahdin as a parent remained virus-free in the field for 2 to 4 years. The remaining 878 clones developed without Katahdin as a parent became 100-percent infected by the vein-banding virus during the 2 to 12 seasons that they were grown in the field.

Marked variation in symptoms, from little or no mottling to moderate or strong mottling of the foliage as well as various degrees of stem

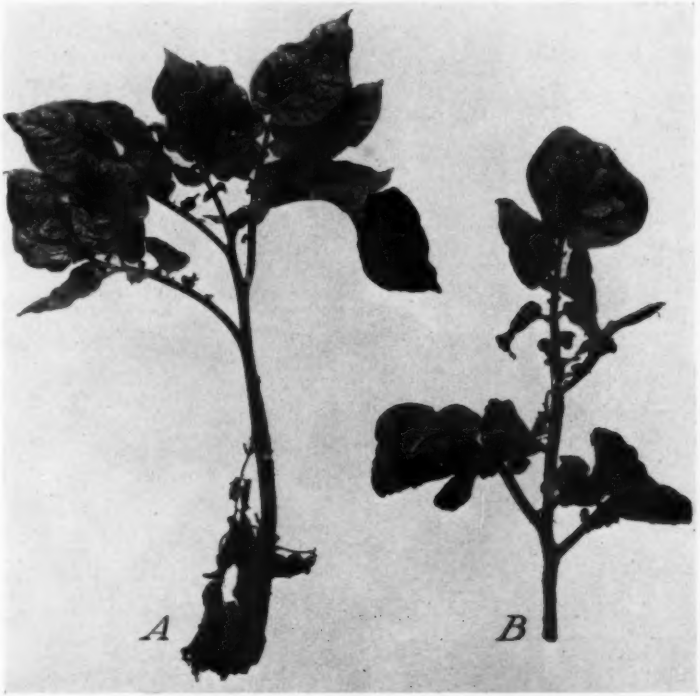

FIGURE 4.—Variation in symptoms produced in seedling potatoes by the vein-banding virus: *A*, Acropetal necrosis in seedling 1425-9; *B*, acronecrosis in seedling 1149-2.

and leaf necrosis, was noted in plants infected by the vein-banding virus only (figs. 3 and 4). These observations are similar to those reported by Schultz et al. (*9*). This variation in symptoms was exhibited by different clones, as the symptoms in all the infected plants of a given clone were uniform. The inoculations into tobacco plants from macerated foliage from each potato plant showed that no X-type virus was associated with this variation. Although some unknown virus that could not be detected by mechanical inoculation into indicator plants of tobacco may have been present, it appears that the variation in symptoms in the different clones was caused by the vein-

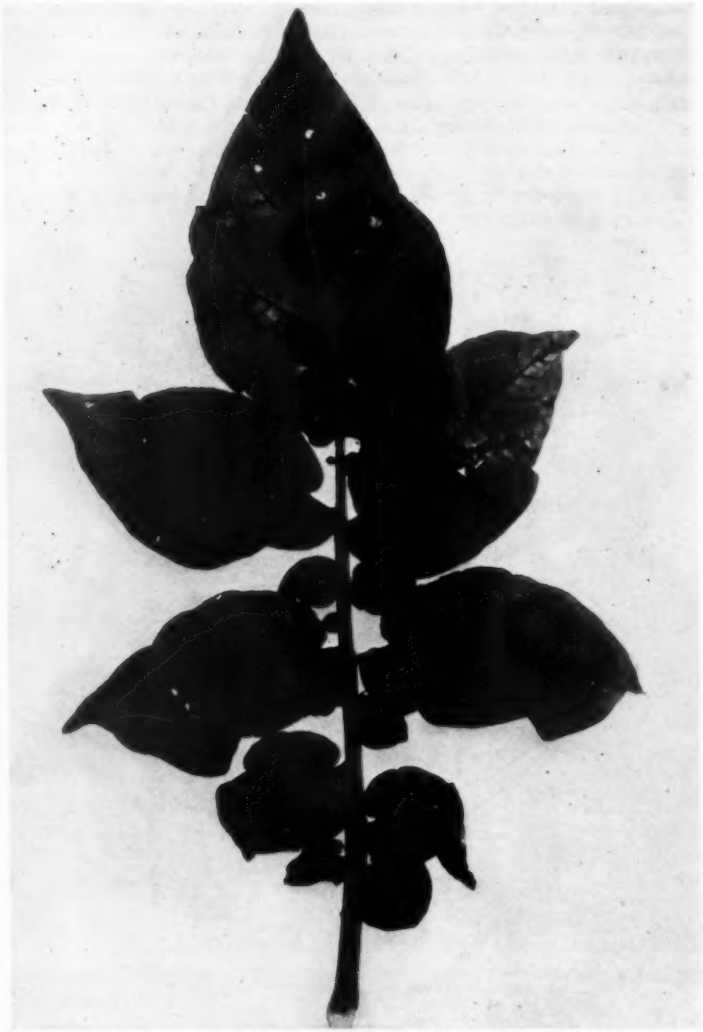

FIGURE 5.—Necrosis in veins in leaf of Chippewa, a symptom of current season infection by the vein-banding virus.

banding virus alone. Curly dwarf symptoms with acropetal necrosis and necrosis in leaf veins (figs. 1 and 5), described in England (*10*) as symptoms associated with Y-virus infection, were noted in many of the clones.

In many of the clones all the plants have shown only a mild to moderate mottling in the foliage and have continued to grow vigorously and produce a good yield in the field even though infected by the vein-

banding virus. This indicates the possibility of producing seedlings that will be highly tolerant to the presence of this virus.

Clones 1028–9, 1066–3, 1145–19, and 1358 showed low percentages of infection by the latent virus, and clones 1069–9, 1154–12 and 1167–7 showed low percentages of infection by the leaf-roll virus, during the 6 to 12 years of field culture. No other viruses appeared in the field plantings.

TESTS WITH COMMERCIAL VARIETIES

Seven commercial varieties of potatoes, and strain no. 41956 furnished by the United States Department of Agriculture, were grown in 1934 and 1935 in the field with the seedling clones to determine the rate of spread of the vein-banding virus to these varieties. Field observations during the early part of the first growing season showed that the Bliss Triumph, Gold Coin, Irish Cobbler, and Russet Burbank were practically free from rugose mosaic and other viruses. Tests in the greenhouse showed that the above four varieties were carriers of the latent virus, while the Chippewa, Katahdin, Warba, and U. S. 41956 were free from both the latent virus and the vein-banding virus when planted in the field in 1934.

Observations were made each growing season to determine the rate of spread of viruses, based on symptom expression. Following harvest in 1935 representative samples of 10 to 40 tubers from each variety were indexed and tested on tobacco plants to determine their virus content (table 3).

TABLE 3.—*The spread of the vein-banding virus to varieties of potatoes grown in the field for 2 years, Pullman, Wash., 1934–35* [1]

Variety	Hills harvested in 1935	Symptoms	Hills affected with vein banding
	Number		*Percent*
Bliss Triumph	125	Rugose mosaic	[2] 100
Chippewa	1,083	Curly dwarf	40
Gold Coin	95	Rugose mosaic	[2] 100
Irish Cobbler	857	do	[2] 100
Katahdin	2,777	Curly dwarf	31
Russet Burbank (strain 1)	1,124	Rugose mosaic	[2] 70
Russet Burbank (strain 2)	778	do	[2] 25
U. S. 41956	33	Mottling	100
Warba	1,083	Curly dwarf	100

[1] Greenhouse tests showed that the Bliss Triumph, Gold Coin, Irish Cobbler, and Russet Burbank stock was 100-percent infected by the latent virus, and that the Chippewa, Katahdin, U. S. 41956, and Warba stock was virus-free, when planted in the field in 1934.

[2] Mixed infection by the vein-banding and latent viruses.

These tests showed that all the varieties are susceptible to field infection by the vein-banding virus. The Katahdin, Chippewa, and Russet Burbank varieties showed some resistance by not becoming 100-percent affected during the 2 years in the field; all other varieties became 100-percent affected under similar conditions of exposure. The Katahdin, Chippewa, and Warba varieties when infected by the vein-banding virus usually show a definite mottling of the foliage (fig. 2, *B*) and necrosis in the veins of the lower leaves (fig. 5) during the season of infection, but the progeny ordinarily shows extreme curly dwarf symptoms in the next season (fig. 1). Of the three varieties, Warba was the most susceptible to infection and showed the most

severe curly dwarf symptoms. Seedling U. S. 41956, which is resistant to the latent virus (*3*, *8*, *9*), is very susceptible to infection by the vein-banding virus, but the infected plants show only a moderate mosaic mottling without necrosis.

Gold Coin, Bliss Triumph, Irish Cobbler, and Russet Burbank plants, which are carriers of the latent virus, show mottling and acropetal necrosis (rugose mosaic) when infected by the vein-banding virus.

Series of 5 to 50 potato plants were inoculated with the vein-banding virus in the greenhouse during the winter months of 1934–36 as follows: six series of Katahdin (totaling 129 plants); two series of Green Mountain (18 plants); two series of Irish Cobbler (10 plants); and one series of Early Rose (10 plants). With each series of inoculated plants a like number of potato plants were left uninoculated as controls. Several sources of the vein-banding virus were used in the inoculation of the different series as follows: Early Rose, Katahdin, seedling 2038, and seedling 2277. The viruses were obtained from potato plants naturally infected in the field and were transferred to tobacco plants in the greenhouse before being inoculated into the potato plants. The four isolations of the vein-banding virus were inoculated into different series of Katahdin but the viruses from Early Rose and seedling 2038 were used in the inoculations into the other varieties.

The potato plants inoculated with the vein-banding virus became infected as follows: Katahdin, 84 percent; Green Mountain, 84 percent; Irish Cobbler and Early Rose, 100 percent. The lower percentage of infection of the inoculated Katahdin and Green Mountain plants indicates a degree of resistance to the vein-banding virus.

Virus-free Katahdin, mechanically inoculated with the vein-banding virus, showed necrosis and curly dwarf symptons, whereas latent-virus-infected Green Mountain, Irish Cobbler, and Early Rose, mechanically inoculated with the vein-banding virus, showed rugose mosaic symptoms. The symptoms produced by mechanical inoculation were similar to those observed in plants naturally infected in the field.

SUMMARY

Field and greenhouse tests with progeny of a single virus-free Early Rose tuber and seedling clones of potatoes have shown that the vein-banding virus spreads very rapidly in the vicinity of Pullman, Wash.

Tests with seedlings developed from crossing and selfing American Wonder, Bovee, Russet Burbank, Early Northern, Early Six Weeks, Irish Cobbler, Jersey Red Skin, Katahdin, Keeper, Late Rose, and McCormick varieties and their seedling progeny have shown that only the Katahdin variety transmitted to progeny resistance to infection by the vein-banding virus.

In tests with the seedling clones marked variation in symptoms, from little or no mottling to moderate or strong mottling of the foliage, as well as various degrees of stem and leaf necrosis, was noted in plants infected by only the vein-banding virus. This variation in symptoms was exhibited by different clones, as the symptoms in all of the infected plants of a given clone were uniform.

Chippewa, Green Mountain, Katahdin, and Russet Burbank showed some resistance to infection by the vein banding virus as

compared with the very susceptible Bliss Triumph, Early Rose, Gold Coin, Irish Cobbler, and Warba.

Chippewa, Katahdin, and Warba, which were virus-free, usually showed dwarfing and extreme necrosis symptoms when infected by the vein-banding virus. Bliss Triumph, Early Rose, Gold Coin, Green Mountain, Irish Cobbler, and Russet Burbank, which were carriers of the latent virus, showed rugose mosaic symptoms when infected by the vein-banding virus.

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